Monitor OIT: Laboratory Tools

H. Henry Li, MD, PhD

Institute for Asthma and Allergy, P.C.

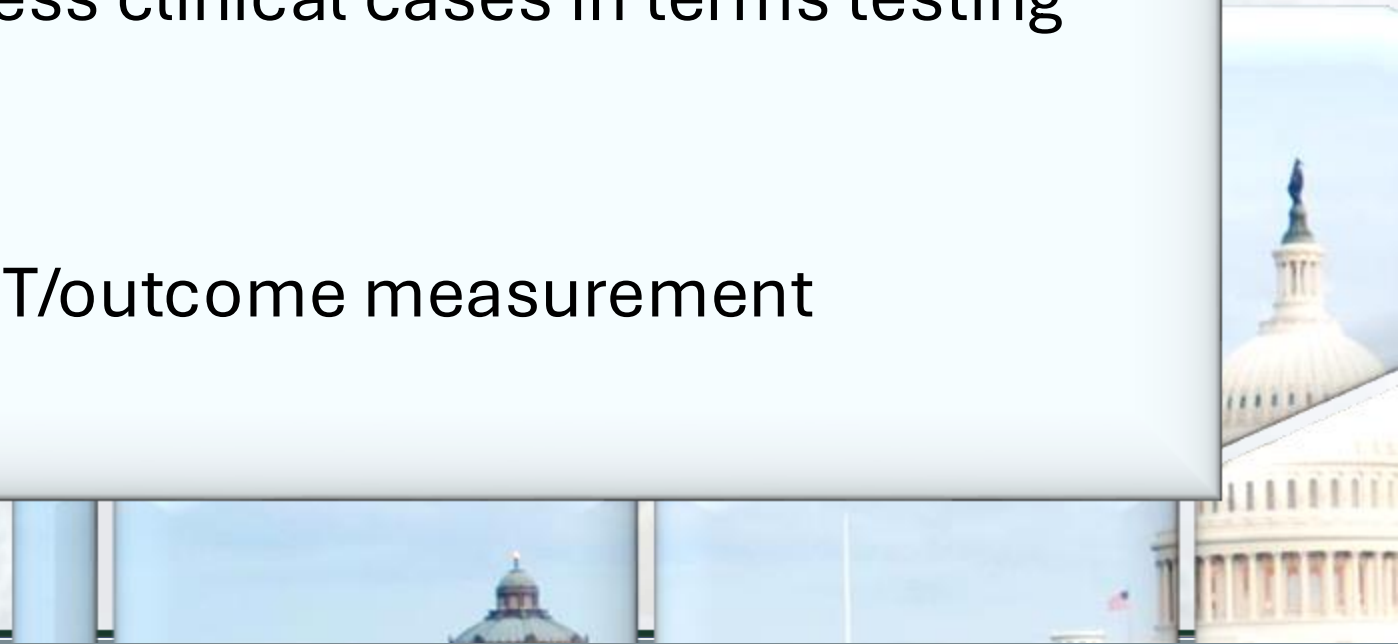
Virant Diagnostics, Inc.

Disclosure: H. Henry Li, MD, PhD

- Employment: Institute for Asthma and Allergy; Virant Diagnostics, etc.
- Speaker/consultation: None related to food allergies
- Clinical trials: PI or Sub-I in multiple clinical trials (food allergy and beyond), none related to the topic for this discussion
- Affiliation: Johns Hopkins University Hospital, George Washington Medical Center; Nankai University School of Medicine.

Learning Objectives

- To familiarize the common food allergy diagnostic tools
- To understand the timing and sequence for food allergy testing
- To use published data to assess clinical cases in terms testing sensitivity and specificity
- To monitor the progress of OIT/outcome measurement



Diagnostic Tests in OIT

- Skin Prick Tests (SPT)
- Specific IgE to extracts (sIgE)
- Specific IgE to components (cIgE)
- Specific IgE to allergen peptides (sIgE-ap)
- Basophil activation test (BAT)
- Mast cell activation test (MAT)
- Specific IgG4 to extracts (sIgG4)
- T cell activation tests or T cell transformation tests (TAT or TTT)
- Food Patch Tests

Skin Prick Tests (SPT) and sIgE

- Detect the presence of specific IgE
- Positive cut-off point
  - ≥ 3mm (neg control) and ≥ 0.35 kU/L
  - High sensitivity and NPV; Low specificity and PPV
- Food specific cut off point with high PPV
- Very useful tools for **initial diagnosis**
- **Not ideal for follow up OIT progress**

Skin Prick Tests vs Specific IgE

	95% PPV			
	SPT (mm)		sIgE (kU/L)	
	≤2 y/o	>2 y/o	≤2 y/o	>2 y/o
Peanut	≥6	≥8		15-34
Tree nuts		≥8		20
Egg	≥4	≥7	2	7
Milk	≥6	≥8	5	15
Sesame		≥8		50 (86)

Du Toit G, et al, Pediatr Allergy Immunol. 2009, 20(4):309-19.
Foong RX,et al. J Allergy Clin Immunol Pract. 2021 Jan;9(1):71-80.

Specific IgE to Allergen Components (cIgE)

- Component-resolved diagnostics (CRD): measures IgE to specific proteins within a food (cIgE)
- Clinically significant sensitization (risk for systemic reaction) compared with clinically irrelevant crossreactivity (oral allergy syndrome)
- Storage protein vs Profilin and Lipid Transfer Protein

Specific IgE to Allergen Components (cIgE)

	Food Allergies	IgE (kU/L)	PPV	Specificity
Casein	Baked Milk	20.2	69%	
Ovomucoid	Baked egg	50	95%	
	Cooked egg	26.6	95%	
	Raw egg	5.21	95%	
Ara h2	Peanut	0.35 – 42.2	90 – 95%	
Ana o3	Cashew	0.16	98%	95%
Cor a9	Hazelnut	1 - 2	79%	100%
Cor a14		0.72 – 47.8	87%	90%
Gly m8	Soy	13.55	89%	74%
Tri a 19	Wheat	0.04 - 41	100%	81%

Foong RX,et al. J Allergy Clin Immunol Pract. 2021 Jan;9(1):71-80.

Specific IgE to allergen peptides (Epitope mapping)

- Identify specific epitopes tha IgE binds within individual allergens
- Clinical relevance and prognosis
- Methodology: Spot Membranes; Microarrays, Luminex-based, etc.
- Peanut >> Milk >> Shellfish > Legumes
  - Predict the likelihood of successful OIT, milk and peanut
  - More useful when interpreted in combination with other assays, sIgE, cIgE, and BAT, etc.
- Not widely used, more studies are needed

Basophil Activation Tests (BAT)

- Functional Assay, virtual challenge test for individual allergens
- CD63 and/or CD203c upregulation upon stimulation
- High Specificity (98.5%, LEAP, LEAP-On, and PAS)
- In a CMA studies:
  - Sensitivity of BAT: 91%
  - Specificity of BAT: 90%
  - Sensitivity of sIgE for milk: 41%

Correlation between OFC (CMA) and BATs

BAT cut-off: percentage of basophils activated by CMP	Se (%)	Spe (%)	PPV (%)	NPV (%)	Efficiency (%)	Correlation factor ϕ with oral challenge	P (χ^2)
2%	97	51	48	97	65	0.463	<0.0001
4%	91	75	63	95	80	0.612	<0.0001
5%	91	82	70	95	85	0.686	<0.0001
6%	91	90	81	96	90	0.752	<0.0001
8%	70	89	74	86	83	0.595	<0.0001
12%	64	92	78	84	83	0.586	<0.0001
15%	45	94	79	79	79	0.480	<0.0001

Allergy, Volume: 66, Issue: 1, Pages: 92-100, First published: 03 December 2010,

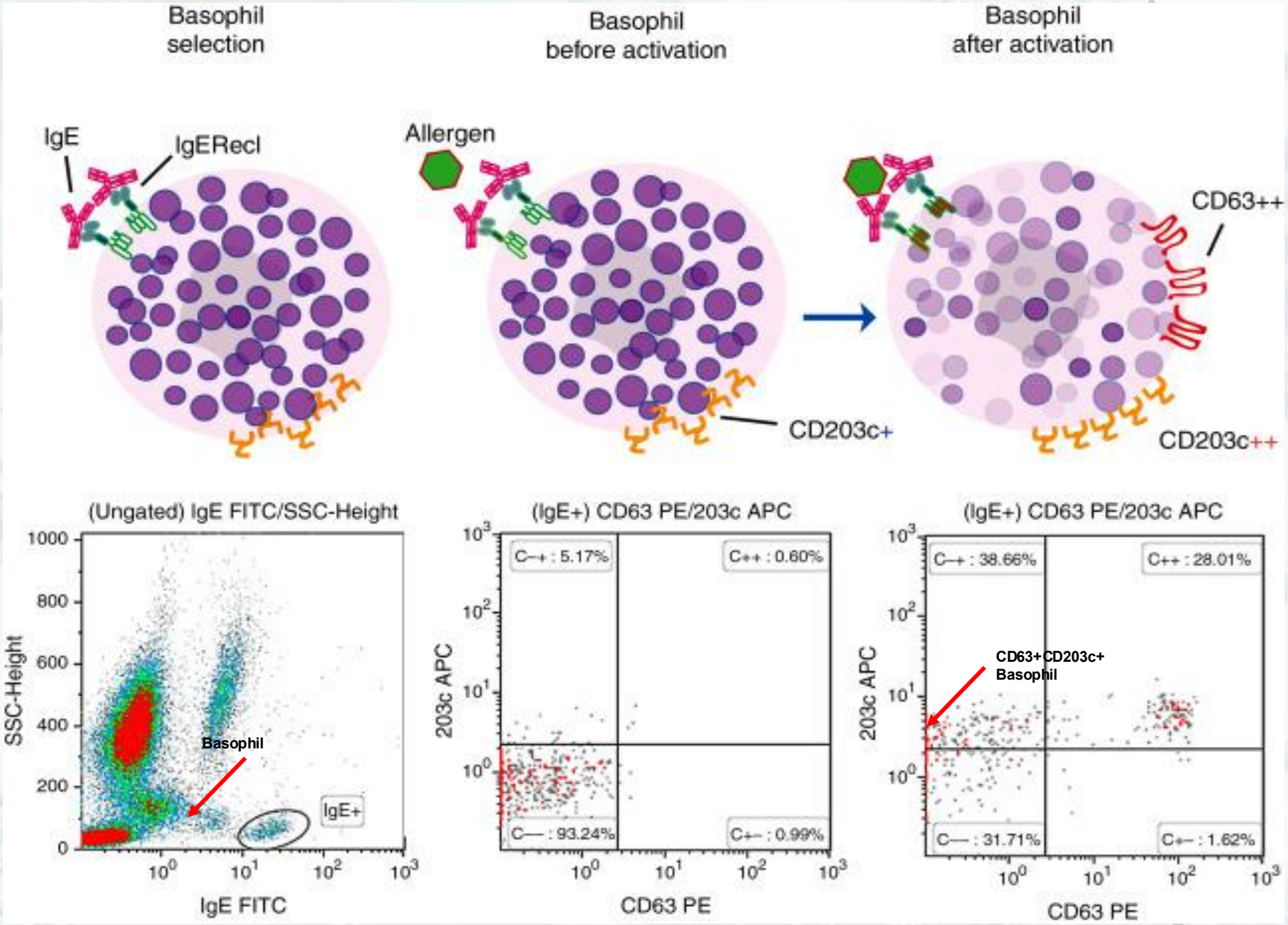
Comparing OFC and Different PN Tests

Tests	PPV (%)	False Positives	False Negative
SPT	75	1	1
sIgE	55	3	3
Ara h2	79	1	2
BAT	86	2	1

Santos AF, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin Immunol. 2014 Sep;134(3):645-52.

VIRANT DIAGNOSTICS

Basophil Activation Test (BAT)

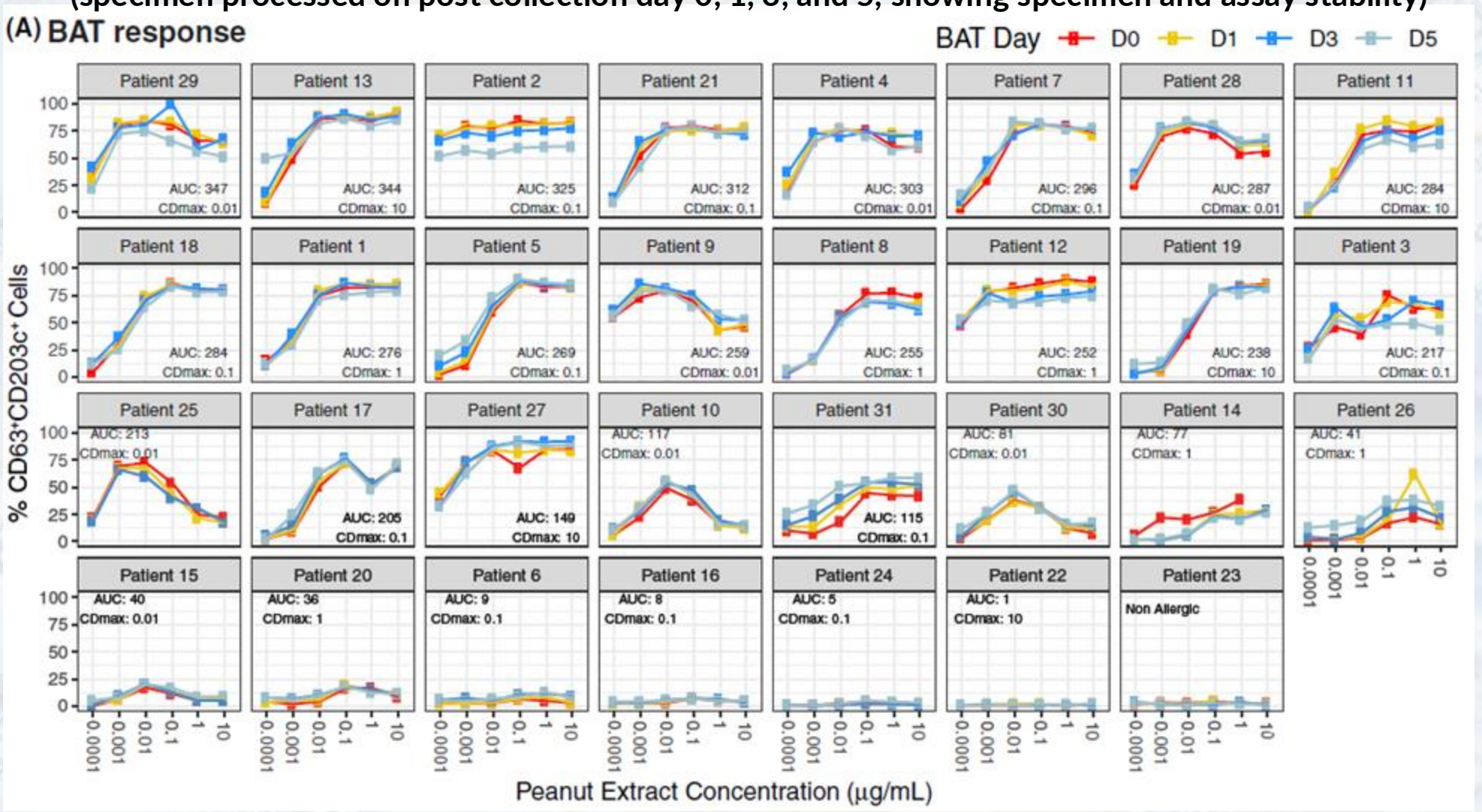


https://doi.org/10.1016/B978-0-12-800756-3.00063-6 (2017, Pages 517-526)

VIRANT DIAGNOSTICS

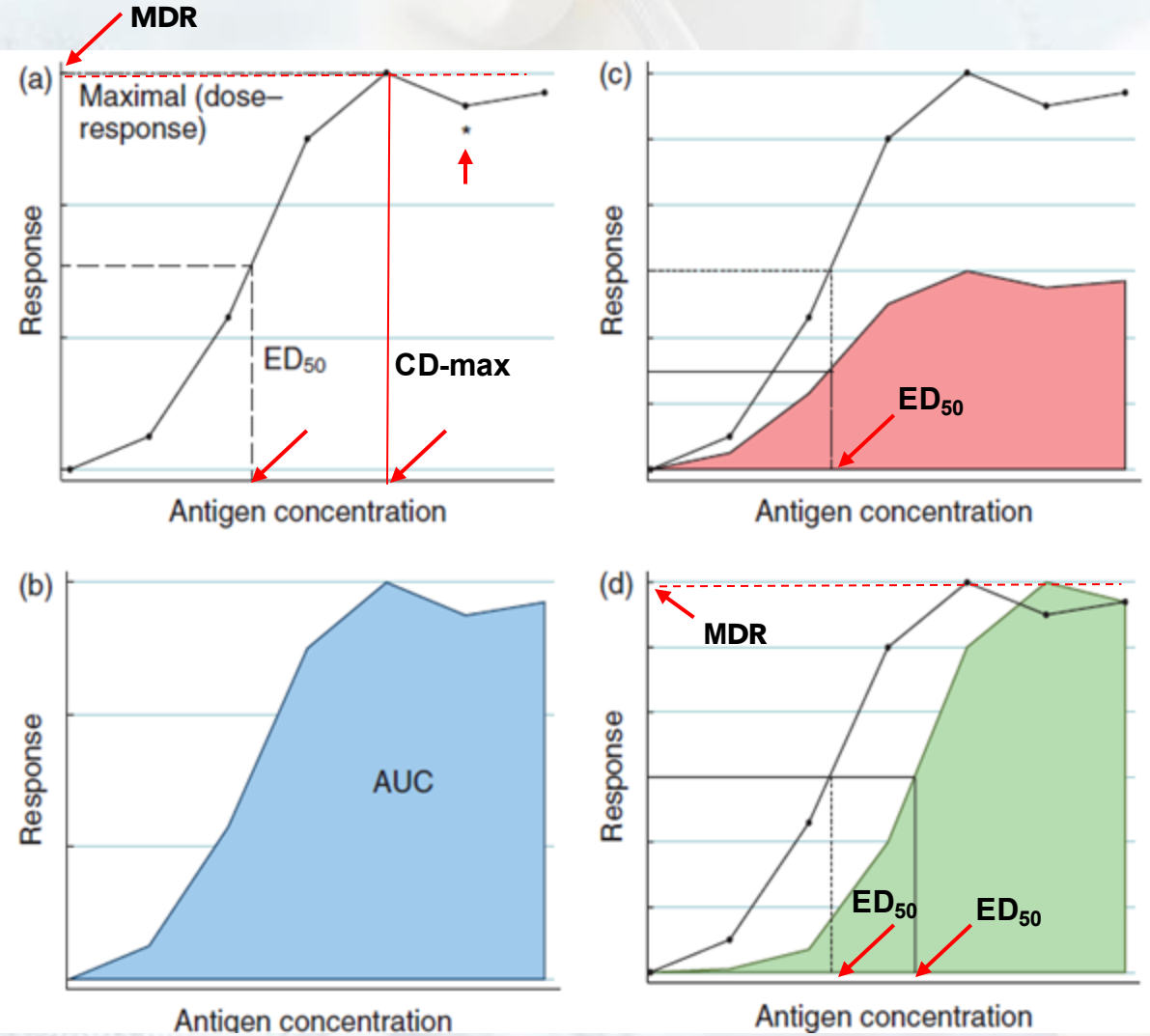
Basophil activation dose-response curve patterns for a total of 31 individual patients

(specimen processed on post collection day 0, 1, 3, and 5, showing specimen and assay stability)



VIRANT DIAGNOSTICS

Quantification of Basophil Activation Test Results



*Refers the supraoptimal part of the dose-response curve.

IgE-mediated basophil activation after stimulated by allergen have different dose–response curve patterns and several proposed parameters to quantify:

MDR: the maximal dose response also known as basophil reactivity (%CD63+ < 5% = negative; antigen-IgE < 5% = non-responder).

ED50: the effective allergen dose at 50% of MDR (also known as basophil sensitivity).

CD-max: the maximal effective allergen dose at MDR.

AUC: considered as the most reliable parameter to quantify basophil activation.

Variations in MDR and AUC but similar ED50 (c).

Variations in ED50, AUC, and CD-max but similar MDR (d).

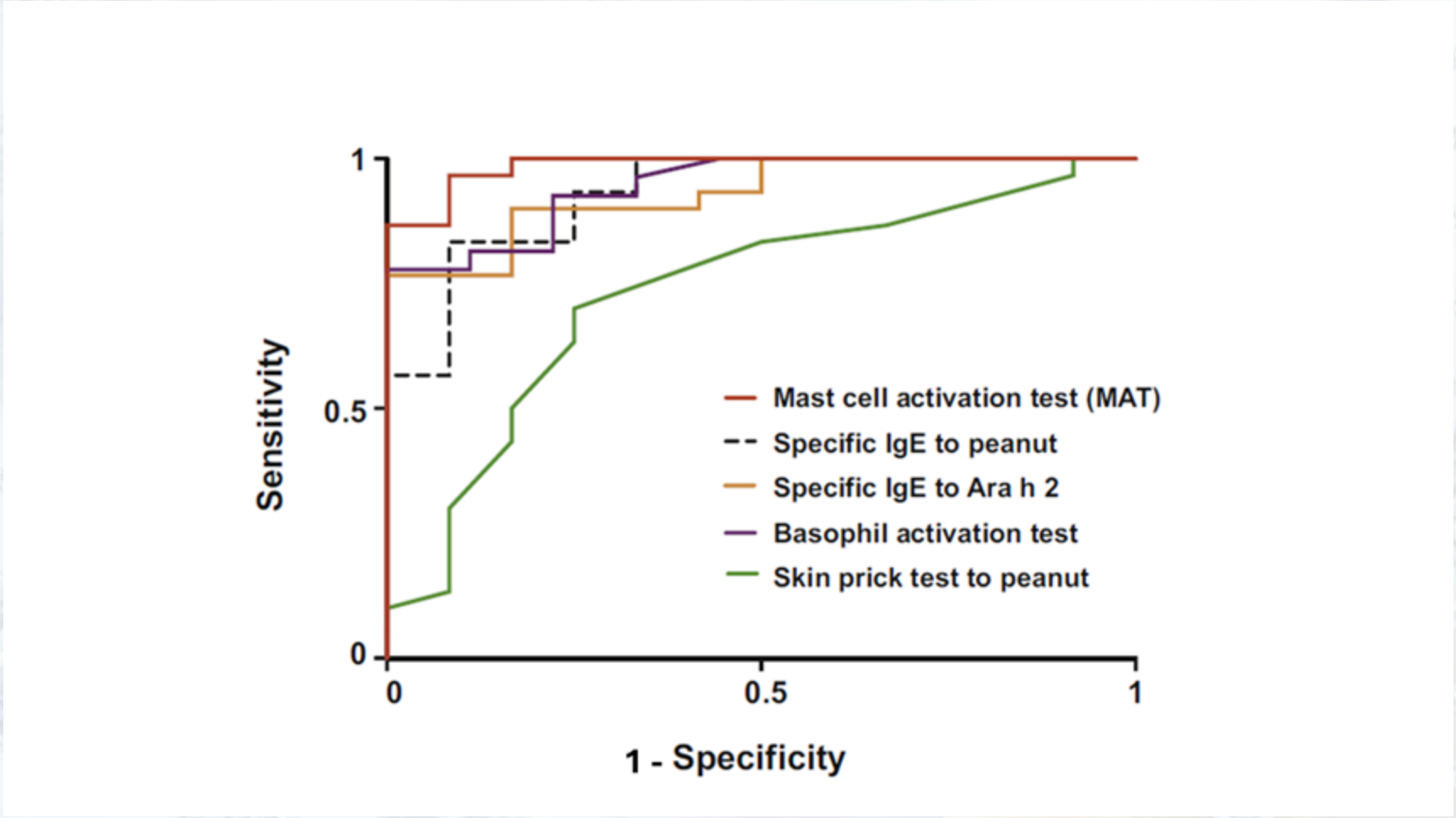
Clinical and Experimental Immunology, 2011:167: 59–66

ROC Curve (Receiver Operating Characterictic Curve)

- **ROC curve**: Commonly applied in the assessment of diagnostic test performance in clinical setting.
- The ROC curve is the plot of the true positive rate (TPR) against the false positive rate (FPR) at each threshold setting.

VIRANT DIAGNOSTICS

ROC Curve comparison between different diagnostic tests for FA



J Allergy Clin Immunol 2018;142:485-96

Virant Diagnostics Protocols

BASOPHIL ACTIVATION MARKERS:

- +CD63 and +CD203c
- IL-3 prime with vs. w/o

BASOPHIL ACTIVATION ANALYSIS:

- % CD63+ and CD203c+ fold change basophils at each allergen concentration
- CD-max: maximal effective concentration
- AUC: area under curve

BASOPHIL ACTIVATION QUANTIFICATION:

- Comprehensive: five allergen concentrations (1, 10, 100, 1000, and 10,000 ng/ml)
- BAT Score: > 5% +CD63 at allergen concentration
  - 5 = 1 ng/ml; 4 = 10 ng/ml; 3 = 100 mg/ml; 2 = 1,000 mg/ml; 1 = 10,000 mg/ml
- AUC / CD-max
- Cumulative weekly dosage (gm)

IMMUNOCAP — PHADIA: SIGE & SIGG4

VIRANT DIAGNOSTICS

Peanut Allergy OIT

Subject INFO						
Total No. of subjects	Age mean (range)	Female (of total) (%)	Age of onset mean (range)	Anaphylaxis (of total individual)	ER Visit (of total individual)	Epi Use (of total individual)
24	13.5 (4-19)	4/24 (16.7%)	4.5 (0.5-10)	15/24	7/24	6/24

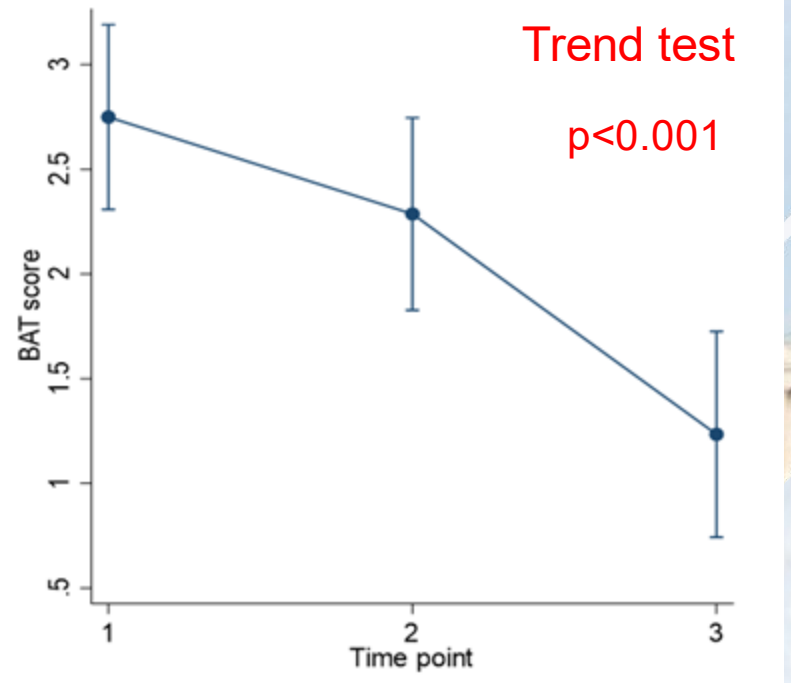
BAT DATA						
BSL BAT mean score	OIT < 6 months BAT mean score	OIT 7-12 months BAT mean score	OIT 13-24 months BAT mean score	OIT > 24 months BAT mean score	No of Responders**	No of Poor Responders***
3.4	2.4	1.6	2.0	0.9	17	4

* 3 subjects were non-compliant patients.
** OIT Responder: decreasing BAT scores with OIT
*** OIT Non-responder: stable or increasing BAT scores with OIT

VIRANT DIAGNOSTICS

Peanut OIT Inhibits Basophil Activation with Decreased BAT Score

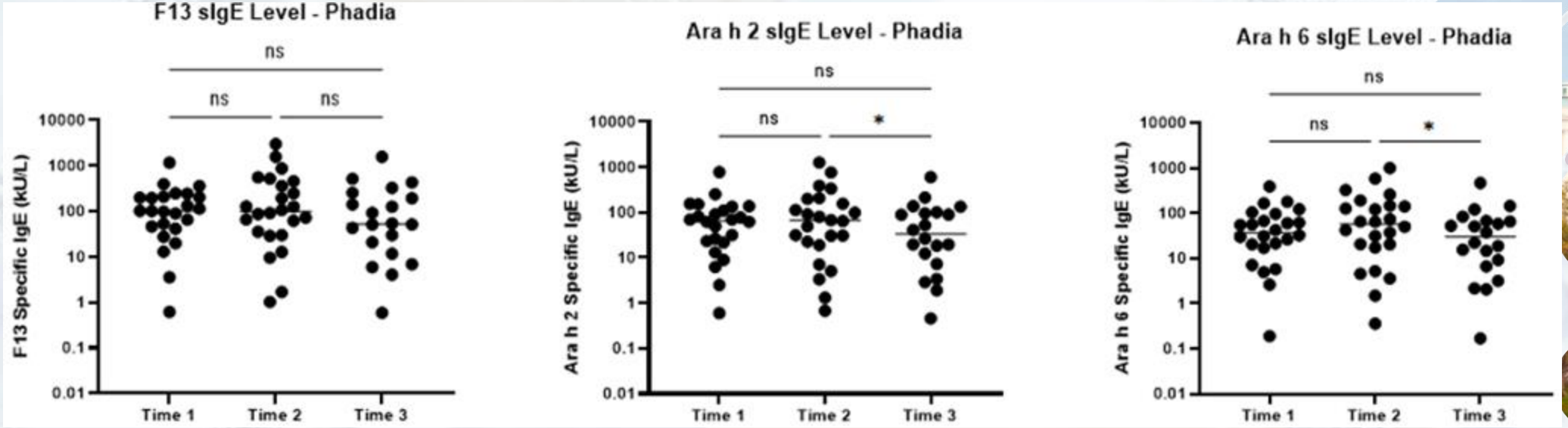
BAT SCORE			
SAMPLE ID	**Time1**	**Time2**	**Time3**
AB24-01	5	3	0
AB24-03	3	2	2
AB24-05	4	2	
AB24-07	3	2	0
AB24-09	3	3	3
AB24-10	4	3	2
AB24-12		5	0
AB24-14	3	3	2
AB24-16	4	3	3
AB24-17	2	2	0
AB24-18	1	1	0
AB24-19	0		3
AB24-20	3	3	2
AB24-27	4	3	1
AB24-28	3	3	
AB24-30	3	2	2
AB24-32	1	2	
AB24-34	2	2	1
AB24-37	3	0	
AB24-38	1		0
AB24-39	3	1	



OIT Duration (months)	Time1	Time2	Time3
mean ± SD	3.4 ± 4.5	13.6 ± 6.3	22.2 ± 7.5
Range	0-15.5	5.0-24.8	8.6-34.0

VIRANT DIAGNOSTICS

Peanut (PNT) and its component sIgE changes depending on OIT dosing phase



PNT sIgE **Ara h2 sIgE** **Ara h6 sIgE**

sIgE AMR: 0.1-100 kAU/L

OIT Duration (months)	Time1	Time2	Time3
mean ± SD	3.4 ± 4.5	13.6 ± 6.3	22.2 ± 7.5
Range	0-15.5	5.0-24.8	8.6-34.0

Trend Test		
Analytes	**Slope**	**p value**
PNT sIgE	17.96	p>0.05
Ara h2 sIgE	-6.27	p>0.05
Ara h6 sIgE	0.19	p>005

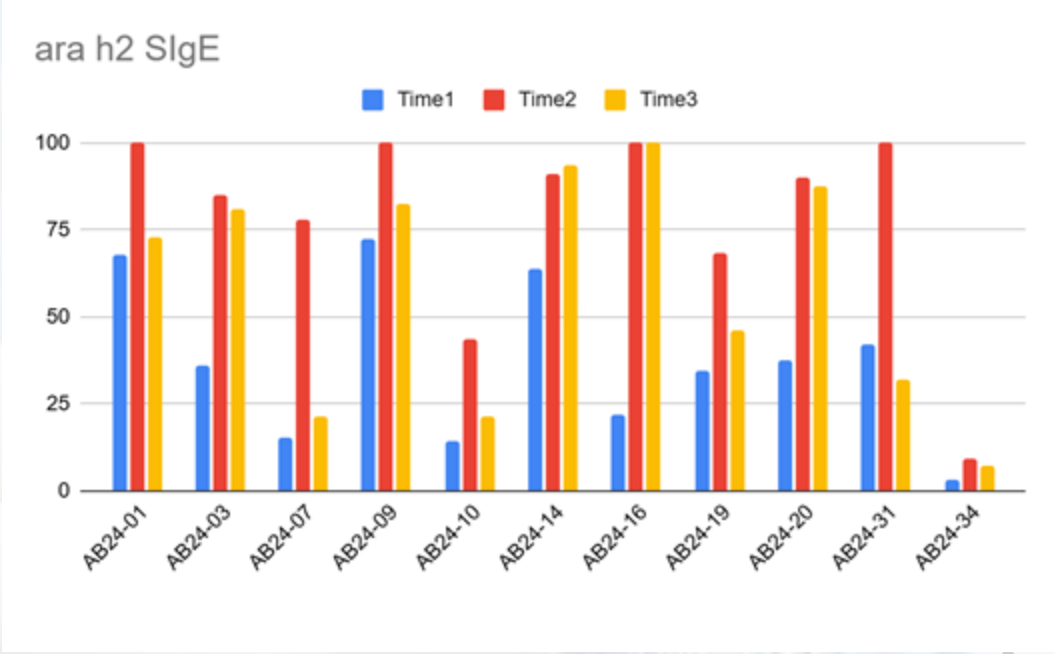
VIRANT DIAGNOSTICS

Evolving Changes in Peanut and its component sIgE Varied with OIT

During the course of OIT, PNT and its component sIgEs were increased compared to prior to the initiation of OIT during the up-dosing phase, and subsequently decreased with the continuation of OIT in maintenance phase for most cases.

Ara h2 sIgE			
Sample ID	**Time1**	**Time2**	**Time3**
AB24-01	67.7	> 100*	72.7
AB24-03	35.9	85.2	81.1
AB24-07	15.2	78.1	21.1
AB24-09	72.3	> 100*	82.6
AB24-10	14.1	43.4	21.1
AB24-14	63.8	90.8	93.7
AB24-16	21.6	> 100*	> 100*
AB24-19	34.4	68.2	46.1
AB24-20	37.4	89.8	87.7
AB24-31	42.1	> 100*	31.9
AB24-34	3.0	9.1	7.4

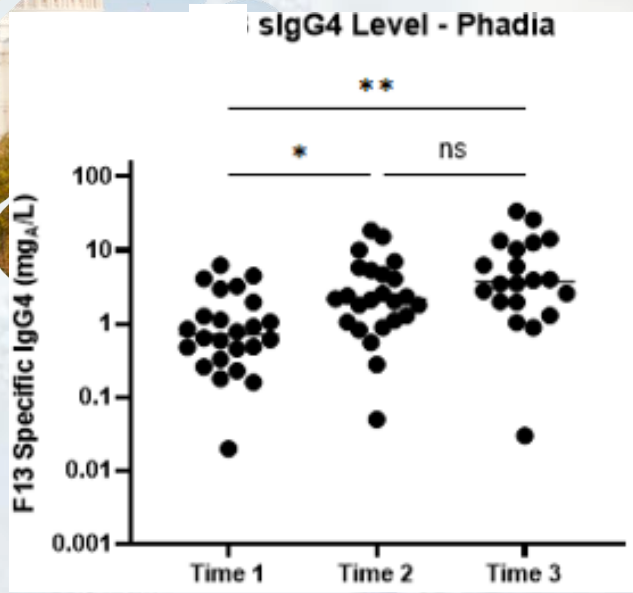
*sIgE AMR: 0.1-100 kUA/L

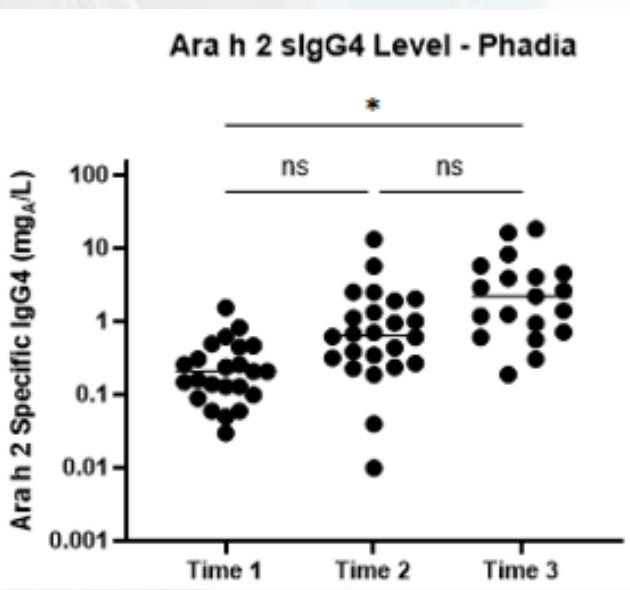


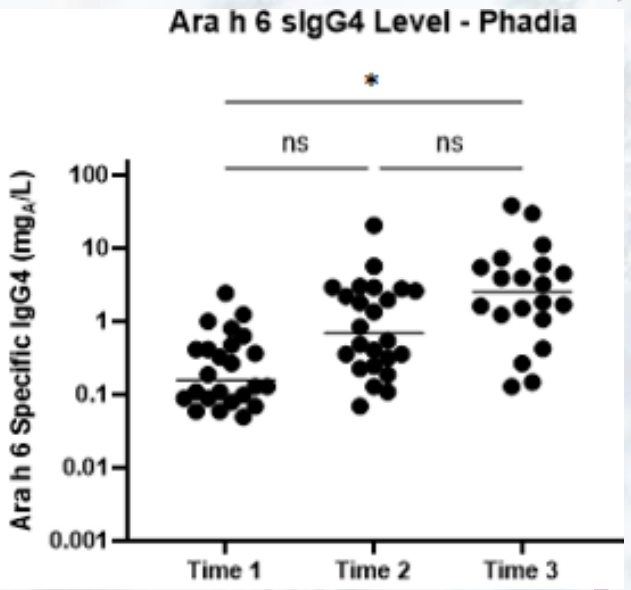
	Time1	**Time2**	**Time3**
OIT Course	baseline (0.0 month)	2.3-9.8 month	11.8-23.9 month

*PNT sIgE and Ara h2 sIgE display similar patterns.

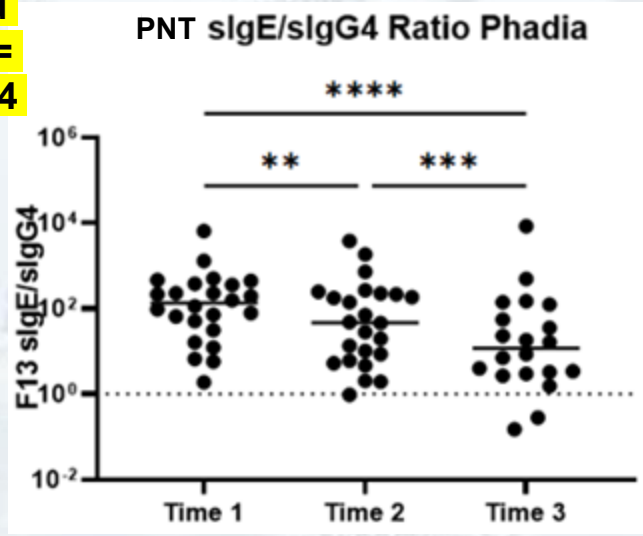
Peanut and Its Components sIgG4 Increases and sIgE/IgG4 Ratio decreases With OIT

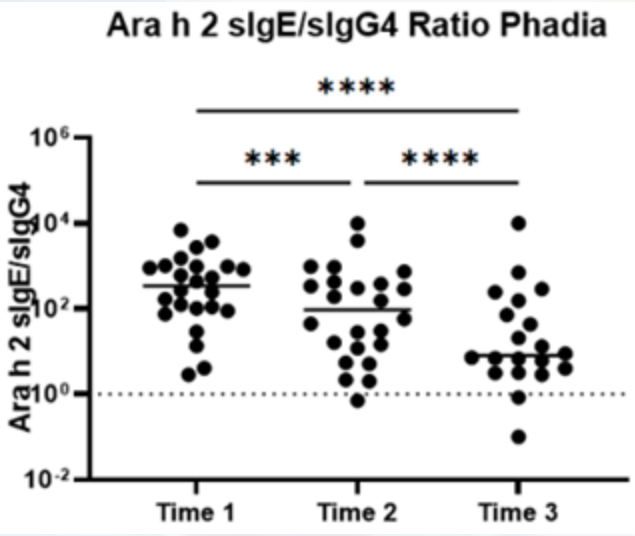


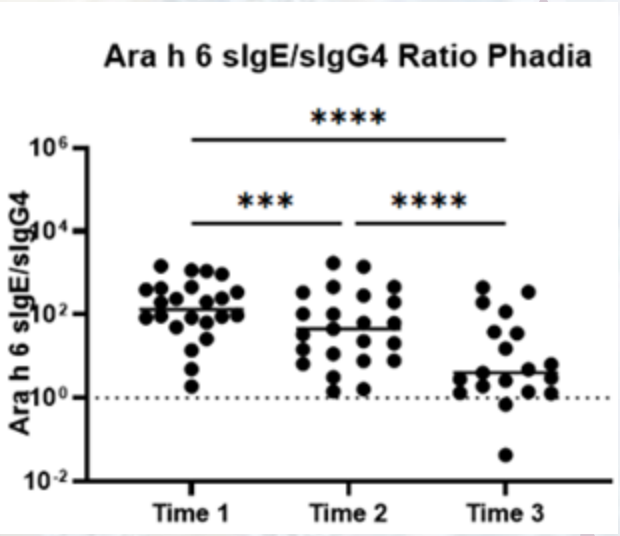




adjusted by sIgE = sIgE/IgG4 ratio



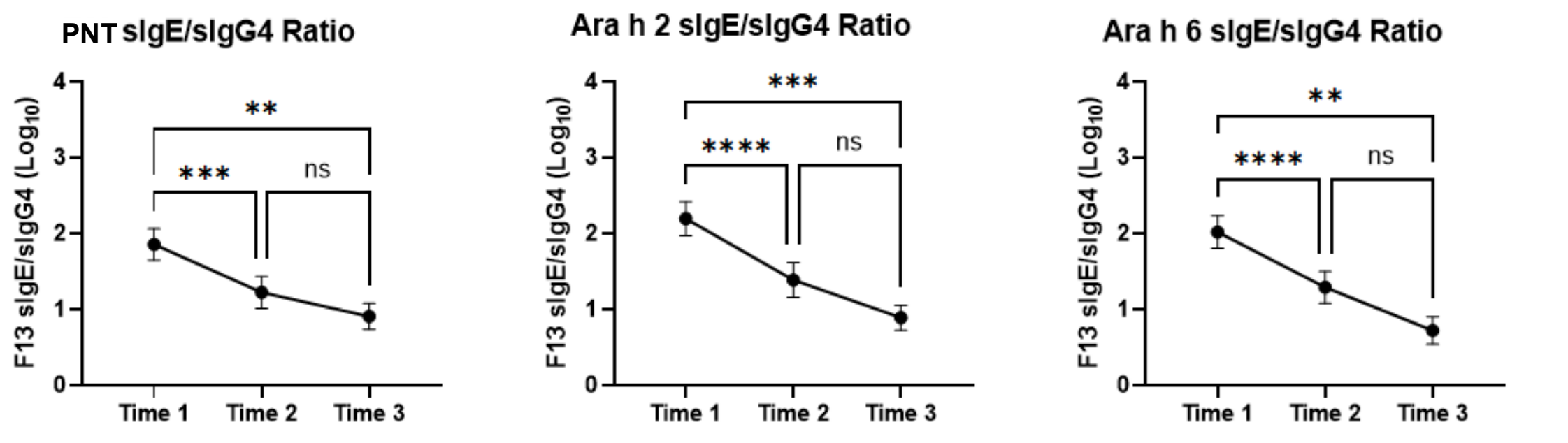




VIRANT DIAGNOSTICS

Significant Decreases of sIgE/sIgG4 Ratios in OIT Responders

(Time3 BAT Score < 2, CD-max = 10,000 ng/ml)



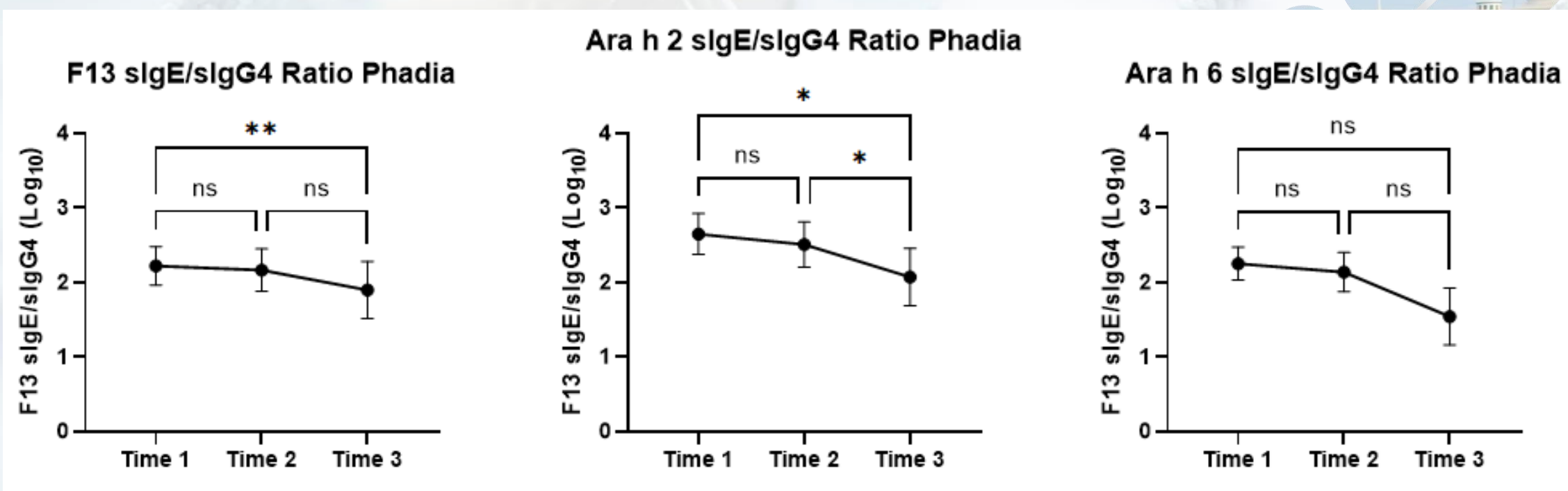
OIT Duration (months)	Time1	Time2	Time3
mean ± SD	3.4 ± 4.5	13.6 ± 6.3	22.2 ± 7.5
Range	0-15.5	5.0-24.8	8.6-34.0

Trend Test		
Analytes	Slope	p value
PNT sIgE/IgG4	-0.48	p<0.01
Ara h2 sIgE/IgG4	-0.66	p<0.001

VIRANT DIAGNOSTICS

No Significant Changes of sIgE/sIgG4 Ratios in OIT Poor Responders

(Time3 BAT Score ≥ 2.0; equals to CD-max ≤ 1000 ng/mL)

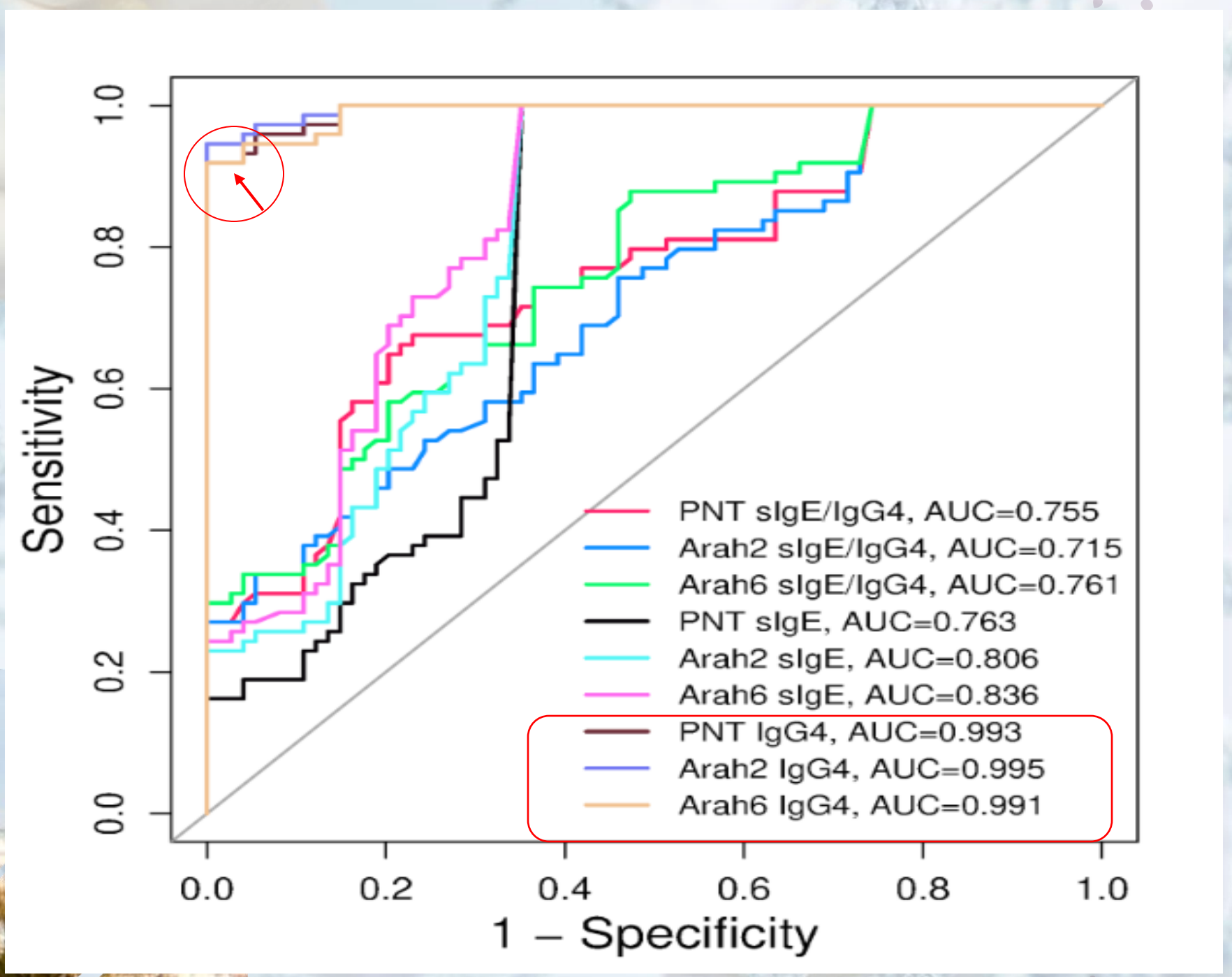


OIT Duration (months)	Time1	Time2	Time3
mean ± SD	3.4 ± 4.5	13.6 ± 6.3	22.2 ± 7.5
Range	0-15.5	5.0-24.8	8.6-34.0

Trend Test		
Analytes	Slope	p value
PNT sIgE/IgG4	-0.15	p>0.05
Ara h2 sIgE/IgG4	-0.28	p>0.05
Ara h6 sIgE/IgG4	-0.33	p>005

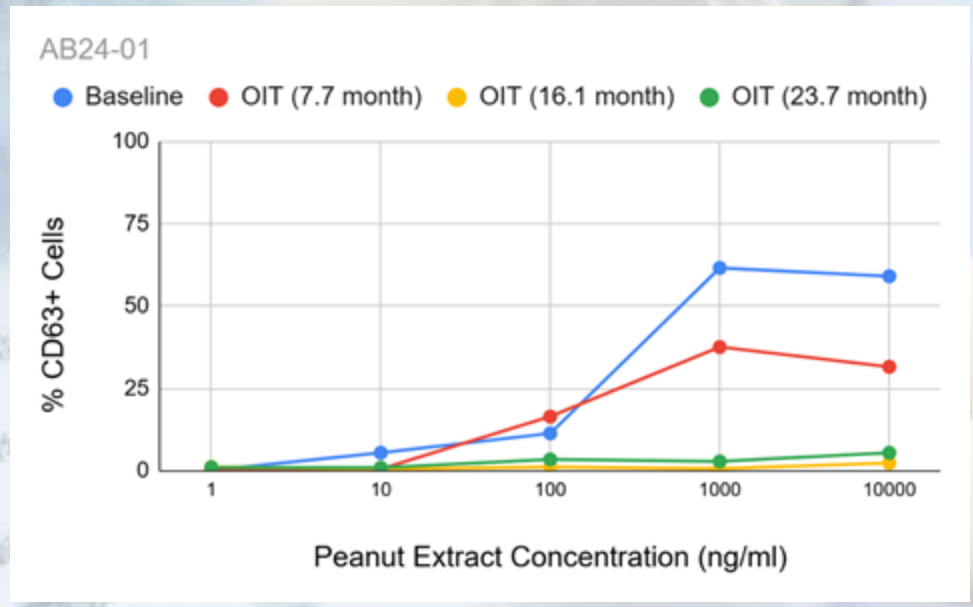
VIRANT DIAGNOSTICS

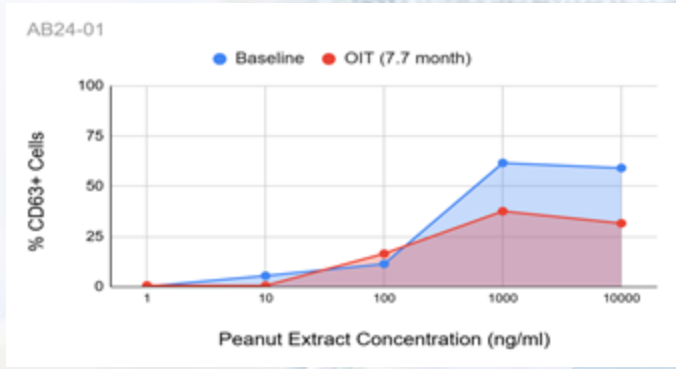
BAT Quantification (AUC) Correlates the Best with PNT and its Component IgG4 With High Sensitivity and Specificity

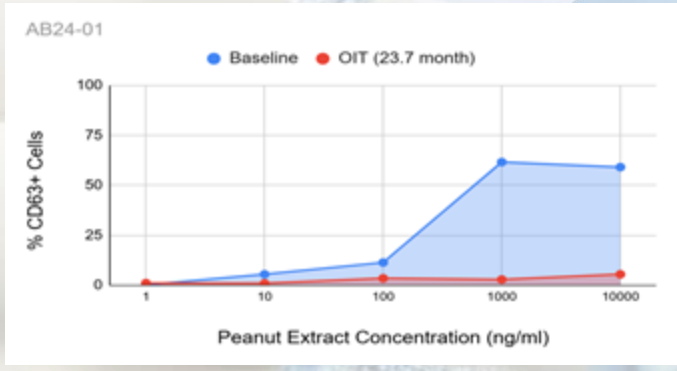


VIRANT DIAGNOSTICS

Basophil Activation Test for A Case of PNT OIT Responder







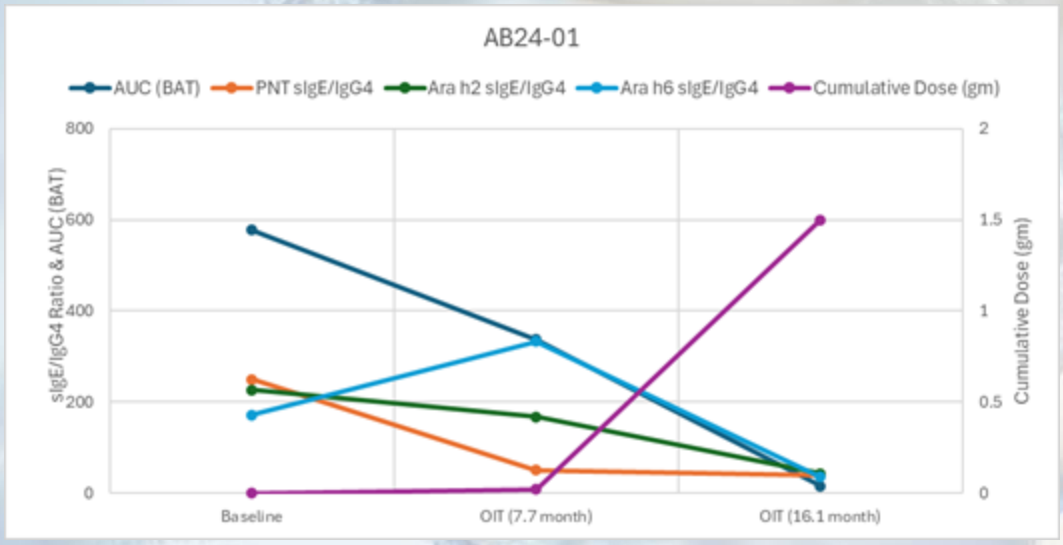
Quantification of BAT

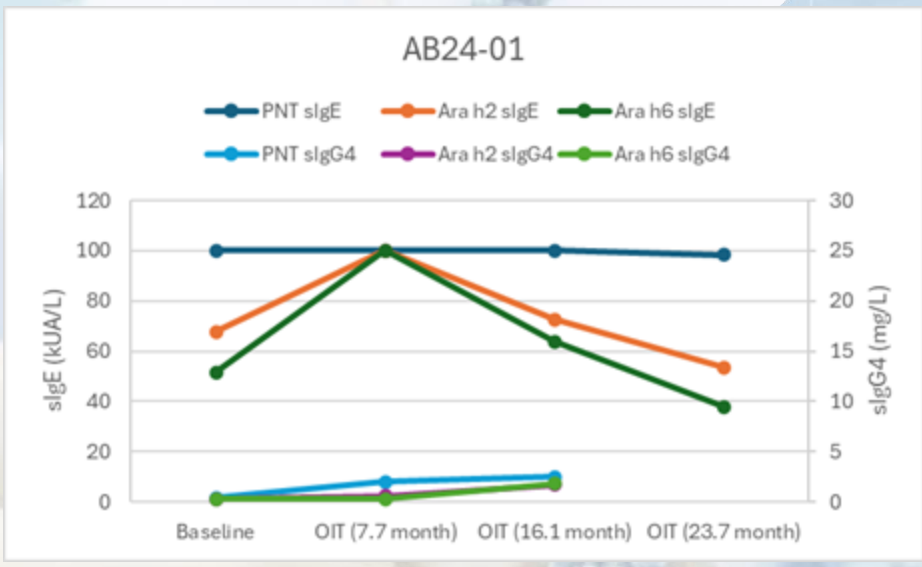
AB24-01	Baseline	OIT (7.7 month)	OIT (16.1 month)	OIT (23.7 month)
Antigen IgE	18.5%	10.3%	11.6%	6.7%
Maximal Dose Response	61.6%	37.6%	2.4%*	5.5%**
CD-max	1000	1000	negative*	10000
AUC	577	337	15	41

* %CD63+ < 5.0%: negative
**Maximal Dose Response %CD63+ Antigen-IgE CD63+CD203c+

** sIgG4 AMR: 0.3-30 mg/L sIgE AMR: 0.1-100 kAU/L

A case of PNT OIT Responder with Decrease in Basophil Reactivity and sIgE & IgG4 Changes





AB24-01	Baseline	OIT (7.7 month)	OIT (16.1 month)	OIT (23.7 month)
AUC (BAT)	577	337	15	41*
PNT sIgE/IgG4	250	50	40**	na
Ara h2 sIgE/IgG4	226	167	43	na
Ara h6 sIgE/IgG4	172	333	35	na
Cumulative wk Dosage (gm)	0	0.02	1.5	4.9

*BAT reactivity (AUC) decrease throughout the course of OIT
**sIgE/IgG4 ratios decrease throughout the course of OIT

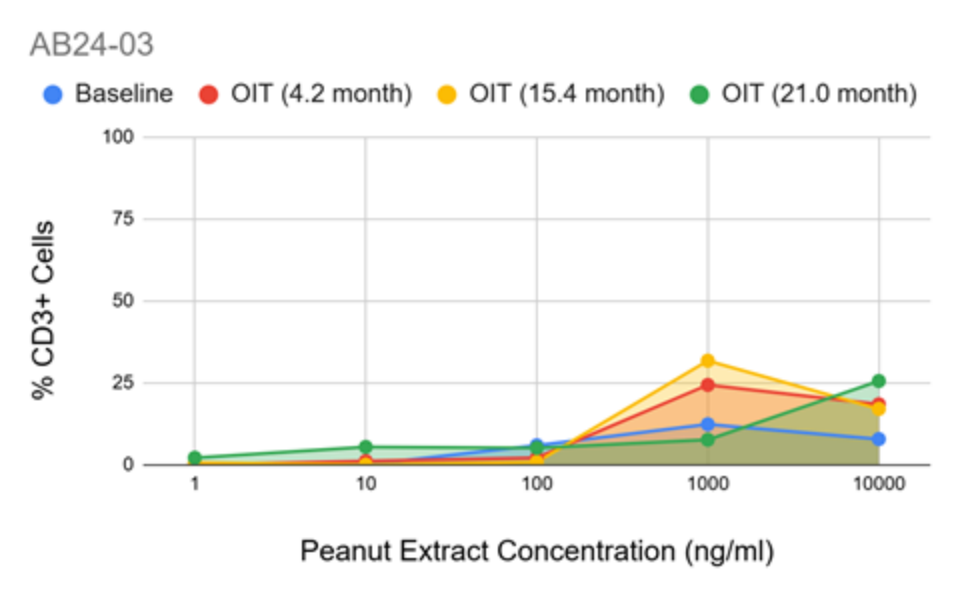
sIgE AMR: 0.1-100 kAU/L; sIgG4 AMR: 0.3-30 mg/L

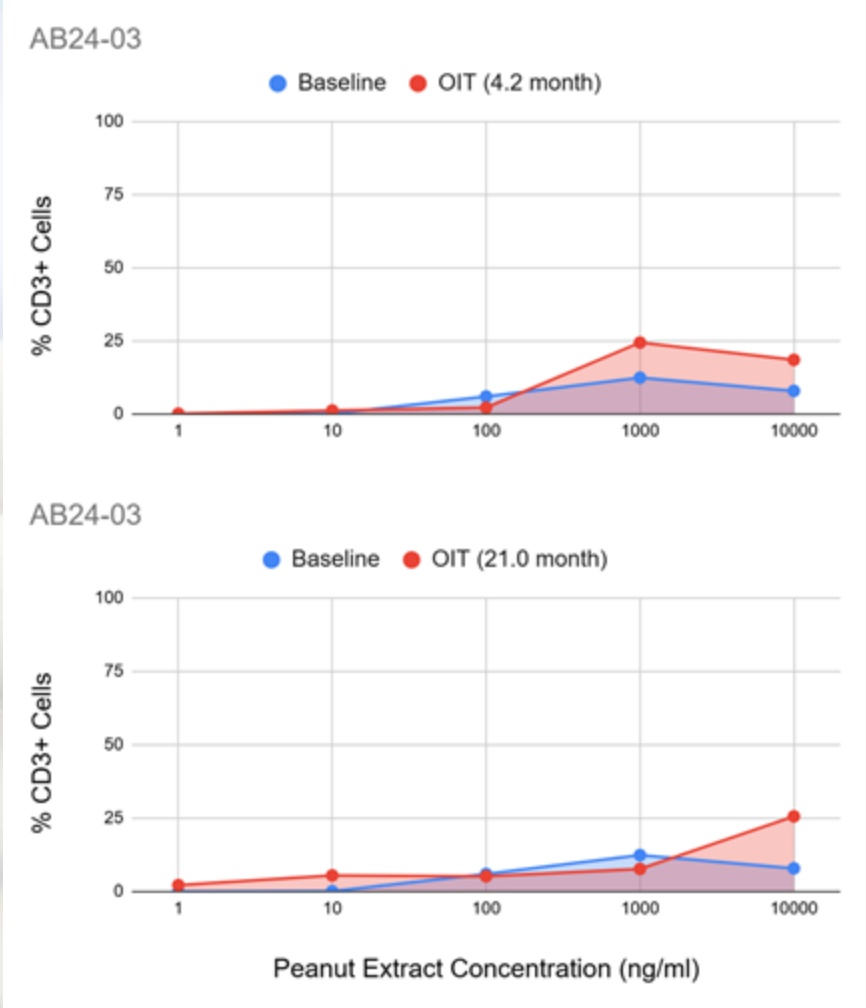
AB24-01	Baseline	OIT (7.7 month)	OIT (16.1 month)	OIT (23.7 month)
PNT sIgE	>100	>100	>100	98.3
Ara h2 sIgE	67.7	>100*	72.7	53.6
Ara h6 sIgE	51.6	>100*	63.8	37.9
PNT sIgG4	0.4	2.0	2.5**	na
Ara h2 sIgG4	< 0.3*	0.6	1.7	na
Ara h6 sIgG4	< 0.3*	0.3	1.8	na

*sIgE increases during up-dosing phase of OIT before decreases over OIT course
**sIgG4 increase throughout the course of OIT

VIRANT DIAGNOSTICS

Basophil Activation Analysis for A Case of PNT OIT Non-Compliance





Quantification of BAT

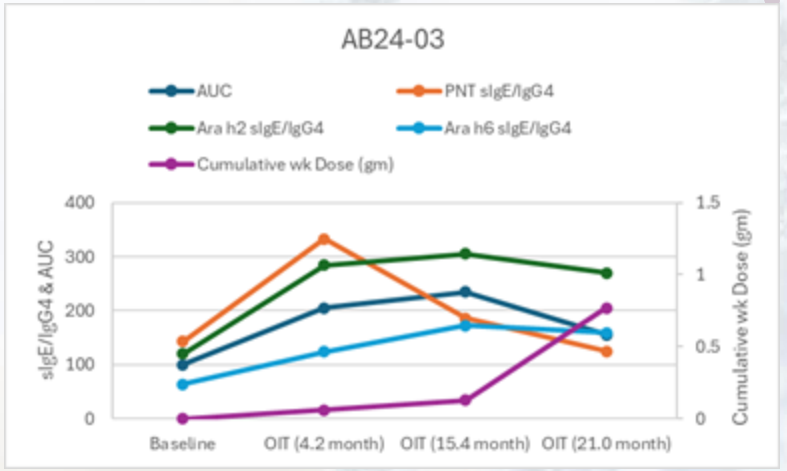
AB24-03	Baseline	OIT (4.2 month)	OIT (15.4 month)	OIT (21.0 month)
Antigen IgE	7.6%	11.8%	19.1%	5.5%
Maximal Dose Response	12.4%	24.4%	31.8%	25.6%
CD-max	1000	1000	1000	10000
AUC	100	205	235	156

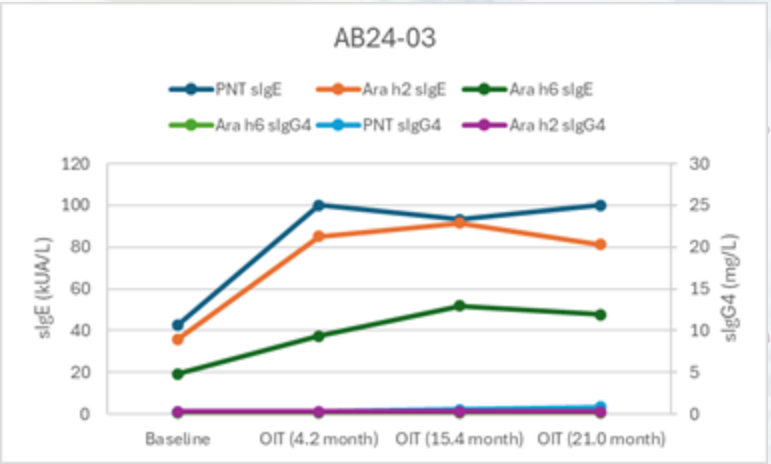
VIRANT DIAGNOSTICS

A case of OIT Non-compliance with Absent sIgG4 Responses and Stable Basophil Reactivity

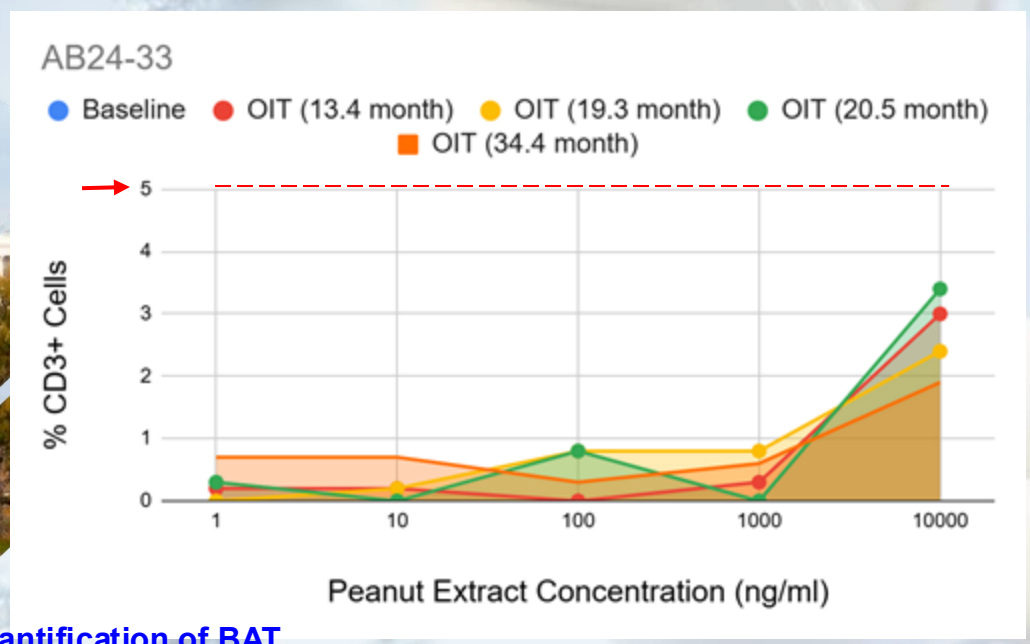
- Onset: 18 month old
- Symptoms: cutaneous and GI
- Severity: h/o ER visit and Epi use
- OIT Course: dose progress slowly and had an aversion to the taste, no overt adverse reaction
- No sIgE decrease throughout OIT course
- No sIgG4 response throughout OIT course

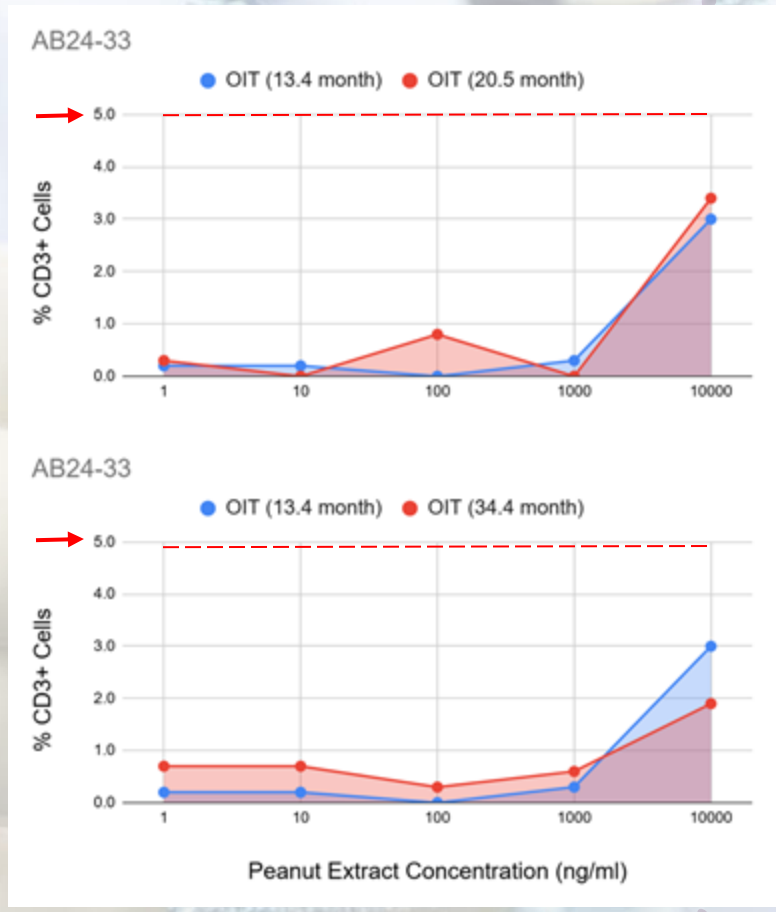
AB24-03	Baseline	OIT (4.2 month)	OIT (15.4 month)	OIT (21.0 month)
AUC (BAT)	100	205	235	156
PNT sIgE/IgG4	143	333	186	125
Ara h2 sIgE/IgG4	120	284	305	270
Ara h6 sIgE/IgG4	64	124	173	159
PNT sIgE	42.8	>100	93.1	>100
Ara h2 sIgE	35.9	85.2	91.6	81.1
Ara h6 sIgE	19.2	37.3	51.8	47.7
PNT sIgG4	< 0.3	< 0.3	0.5	0.8*
Ara h2 sIgG4	< 0.3	< 0.3	< 0.3	< 0.3*
Ara h6 sIgG4	< 0.3	< 0.3	< 0.3	< 0.3*
Cumulative wk Dose (gm)	0	0.06	0.13	0.77





Basophil Activation Analysis for A BAT Non-Responder





Quantification of BAT

AB24-33	Baseline	OIT (13.4 month)	OIT (19.3 month)	OIT (20.5 month)	OIT (34.4 month)
Antigen-IgE*	na	2.1%*	1.0%*	0.3%*	8.0%
Maximal Dose Response	na	3.0%**	2.4%**	3.4%**	1.9%
CD-max	na	10000	10000	10000	10000
AUC	na	15	15	16	12

*antigen-IgE CD63+CD203c+: < 5% = non responder (w/o IL-3 prime)

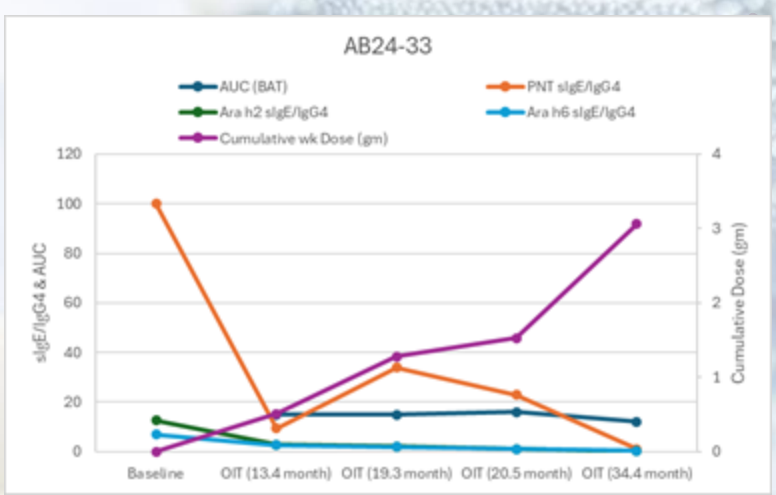
**Maximal Dose Response %CD63+ > antigen-IgE CD63+CD203c+

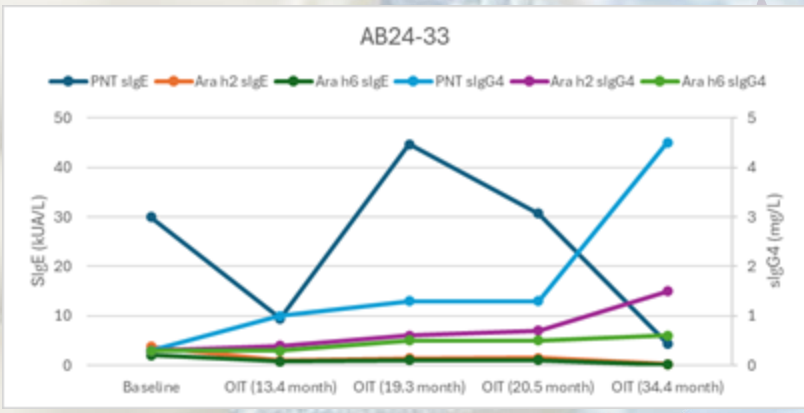
VIRANT DIAGNOSTICS

A Case of BAT Non-responder With sIgE & IgG4 Changes With OIT

- Onset: 8 month
- Symptoms: mainly GI
- Severity: no ER visit or Epi use
- OIT Course: no adverse reactions
- BAT non-responder: anti-IgE < 5.0%
- sIgE decreases
- sIgG4 increases

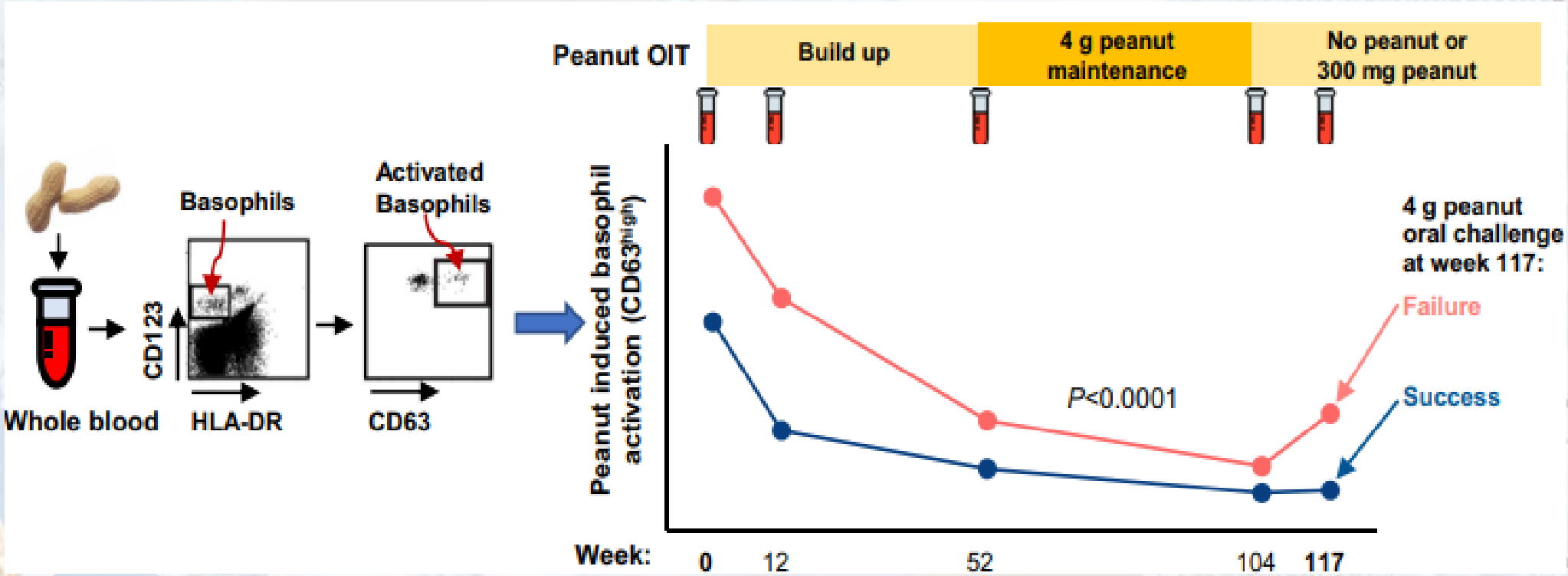
AB24-33	Baseline	OIT (13.4 month)	OIT (19.3 month)	OIT (20.5 month)	OIT (34.4 month)
AUC	na	15	15	16	12
PNT sIgE/IgG4	100	9.5	34.4	23.6	1.0
Ara h2 sIgE/IgG4	12.7	2.8	2.2	1.0	0.2
Ara h6 sIgE/IgG4	7.0	2.7	2.0	1.0	0.3
PNT sIgE	30.0	9.5	44.7	30.7	4.4
Ara h2 sIgE	3.8	1.0	1.4	1.5	0.3
Ara h6 sIgE	2.1	0.8	1.1	1.1	0.2
PNT sIgG4	< 0.3	1.0	1.3	1.3	4.5
Ara h2 sIgG4	< 0.3	0.4	0.6	0.7	1.5
Ara h6 sIgG4	< 0.3	0.3	0.5	0.5	0.6
Cumulative wk Dose (gm)	0	0.51	1.28	1.53	3.06





VIRANT DIAGNOSTICS

Sustained Desensitization With OIT Associated With Low Basophil Activation

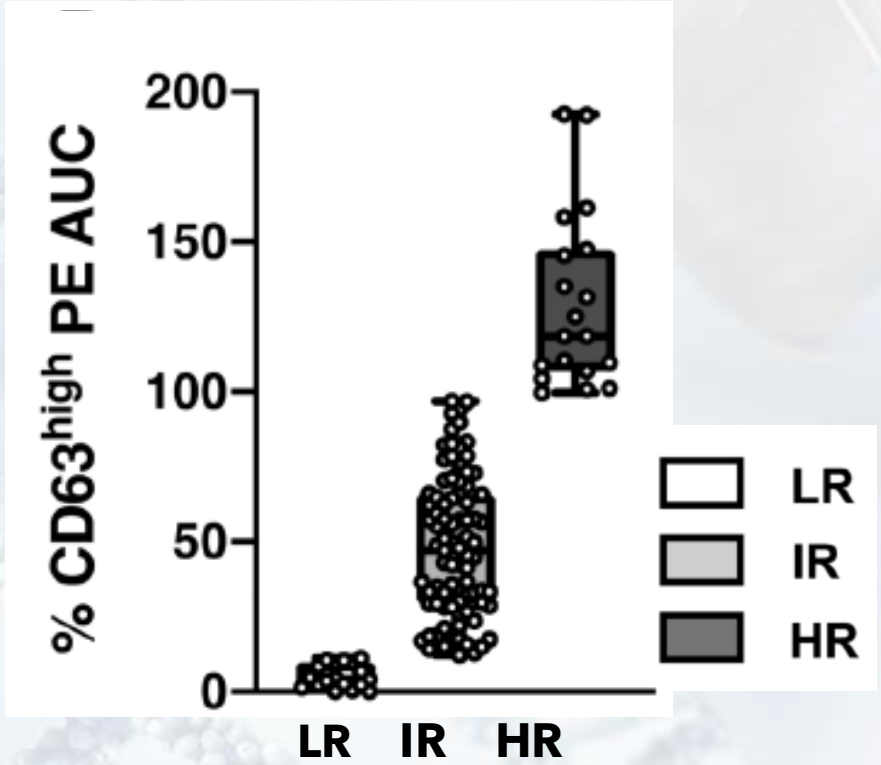


Tsai M, et al. J Allergy Clin Immunol 2020;145:885

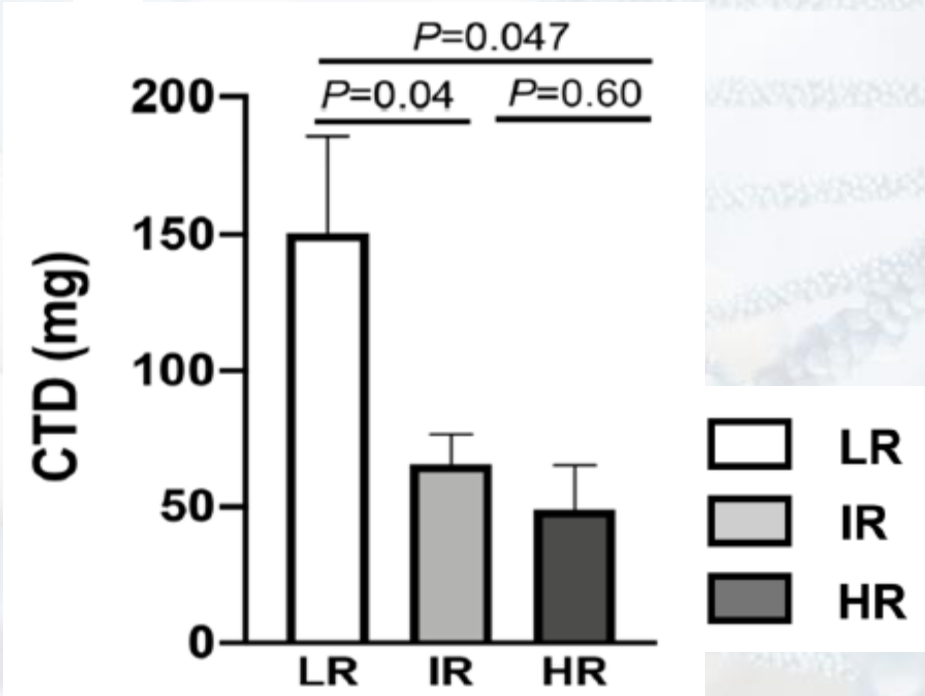
VIRANT DIAGNOSTICS

Can baseline BAT predicts OIT outcome ?

Basophil poor responders exhibit lower peanut sIgE and better treatment outcome



Cumulative tolerated peanut challenge dose (CTD) at Week 0



Tsai M, et al. J Allergy Clin Immunol 2020;145:885

VIRANT DIAGNOSTICS

Low basophil reactivity at week 0 (baseline) associated with sustained effectiveness of OIT

Basophil status (week 0)	Peanut 0+Peanut 300					
	Wk 117 DBPCFC			Wk 156 DBPCFC*		
	Pass	Fail	*P* value	Pass	Fail	*P* value
Non/Low Responder	10 (91%)	1 (9%)	0.001	6 (55%)	5 (45%)	0.08
Intermediate responder	28 (48%)	30 (52%)		13 (22%)	45 (78%)	
High responder	2 (17%)	10 (83%)		2 (17%)	10 (83%)	

Tsai M, et al. J Allergy Clin Immunol 2020;145:885

Conclusion: Food allergy testing

- Clinical history: Very important
- Initial screening, establishing diagnosis
  - SPT, sIgE, and cIgE should be considered as first and second line tests
  - Understand the pros and cons of each test methods
- BAT, IgG4, particularly IgE/IgG4 ratio are great biomarkers for monitoring the progress of OIT


Thankyou