Basophil Activation Tests

Douglas H Jones, FAAAAI, FACAAI

Jumbo Shrimp… more than an oxymoron

BAT: What does it measure?

BAT focuses on the basophil population at a single cell level using flow cytometry and assesses the activation state of these cells before and after stimulation with allergens or controls.

Principle of the assay

Stimulation of basophils with an allergen leads to appearance of several surface markers that can be measured by flow cytometry. We will refer to them as "inducible biomarkers" as they are induced after incubation of the whole blood with the allergen.

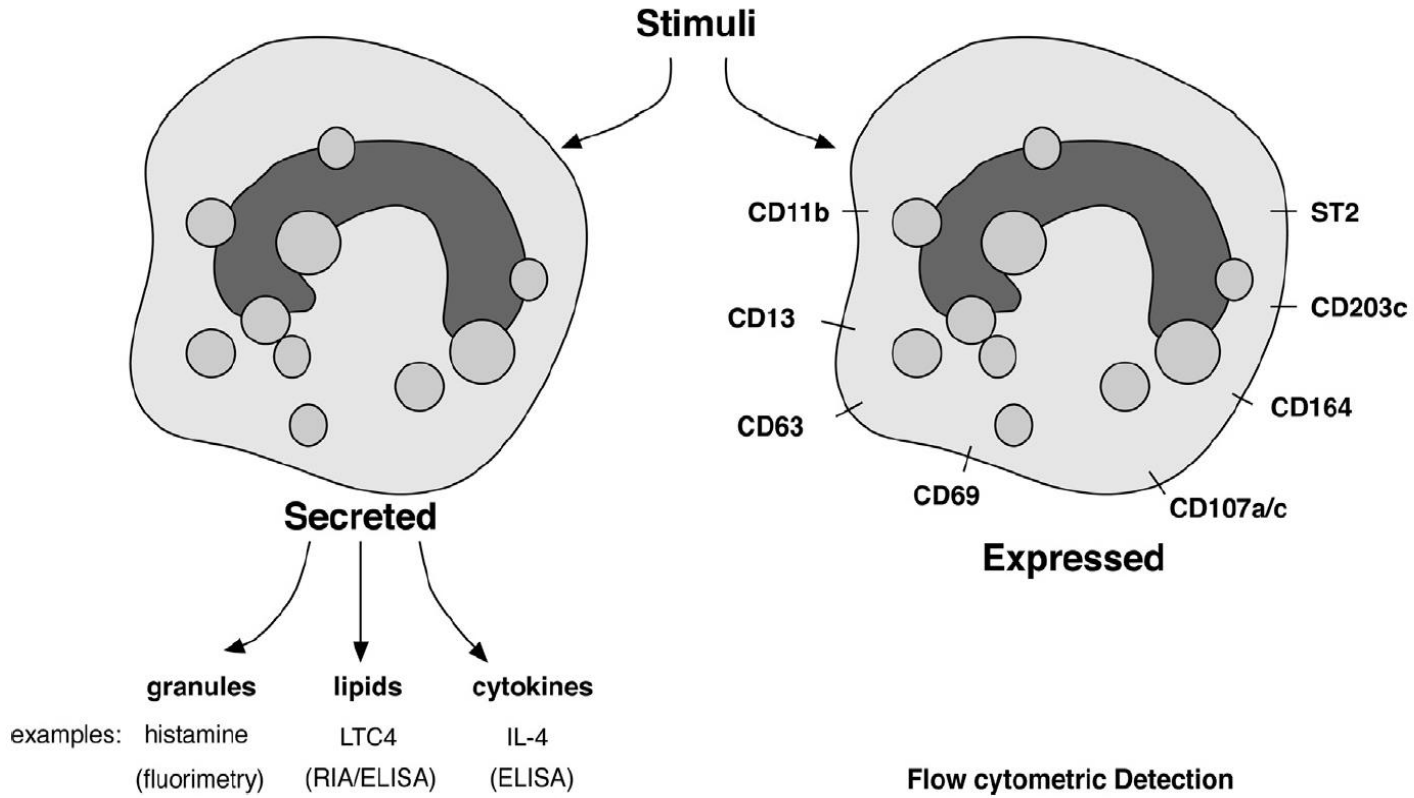
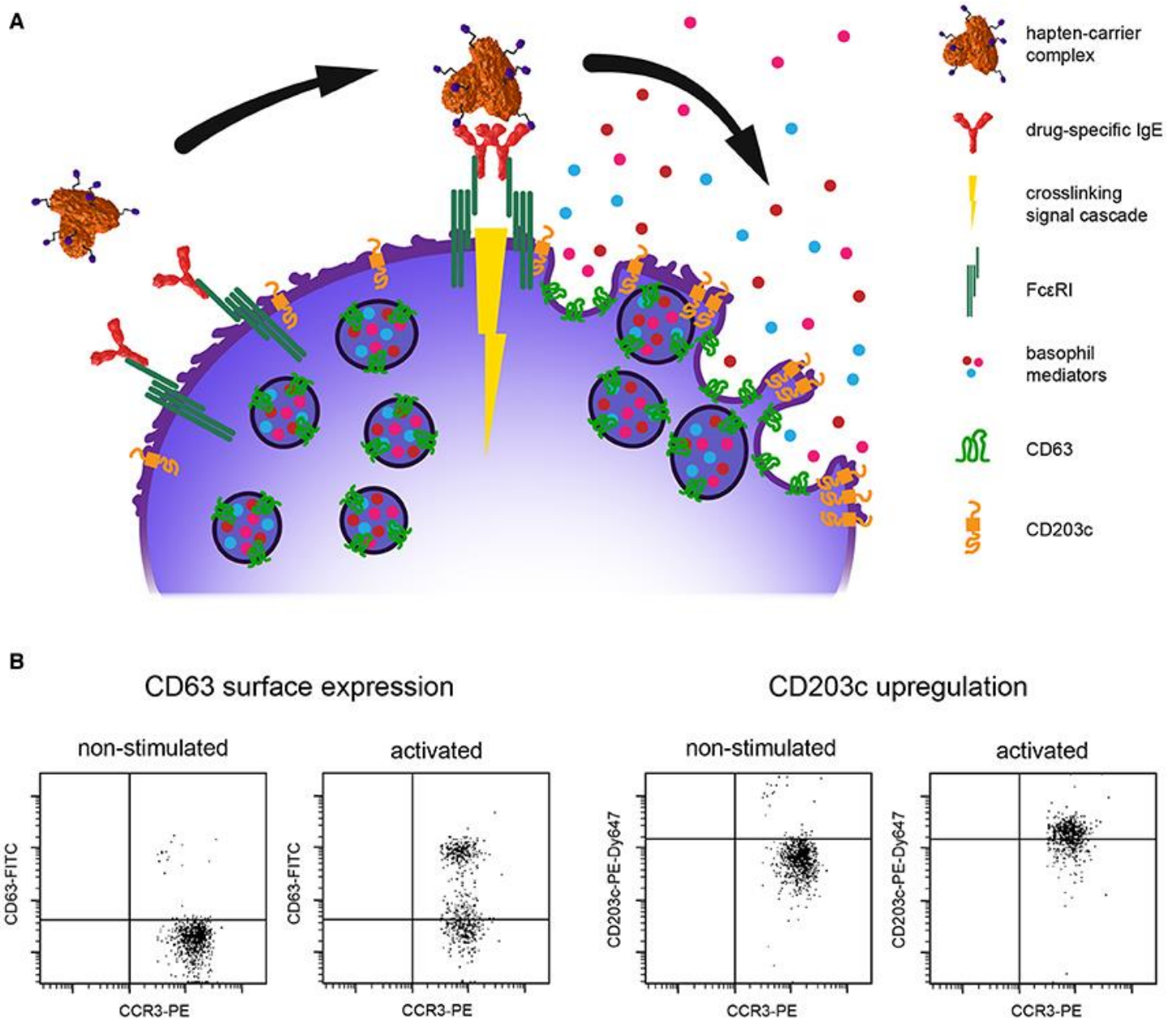


FIG 1. The 2 predominant methods to assess activation of basophils: (1) measurement of mediator release or (2) increased or newly expressed cell-surface proteins.

Inducible markers on basophils:

- ***CD63:*** This antigen is mainly associated with membranes of intracellular vesicles. Cell surface expression may be induced when intracellular vesicle fuse with the cell membrane – hence an indication of cell degranulation.

- ***CD203c:*** ectoenzymes that are involved in hydrolysis of extracellular nucleotides. These ectoenzymes possess ATPase and ATP pyrophosphatase activities and are type II transmembrane proteins. This protein has also been used in conjunction with CD63 as a marker for activated basophils in the basophil activation test for IgE mediated allergic reactions.



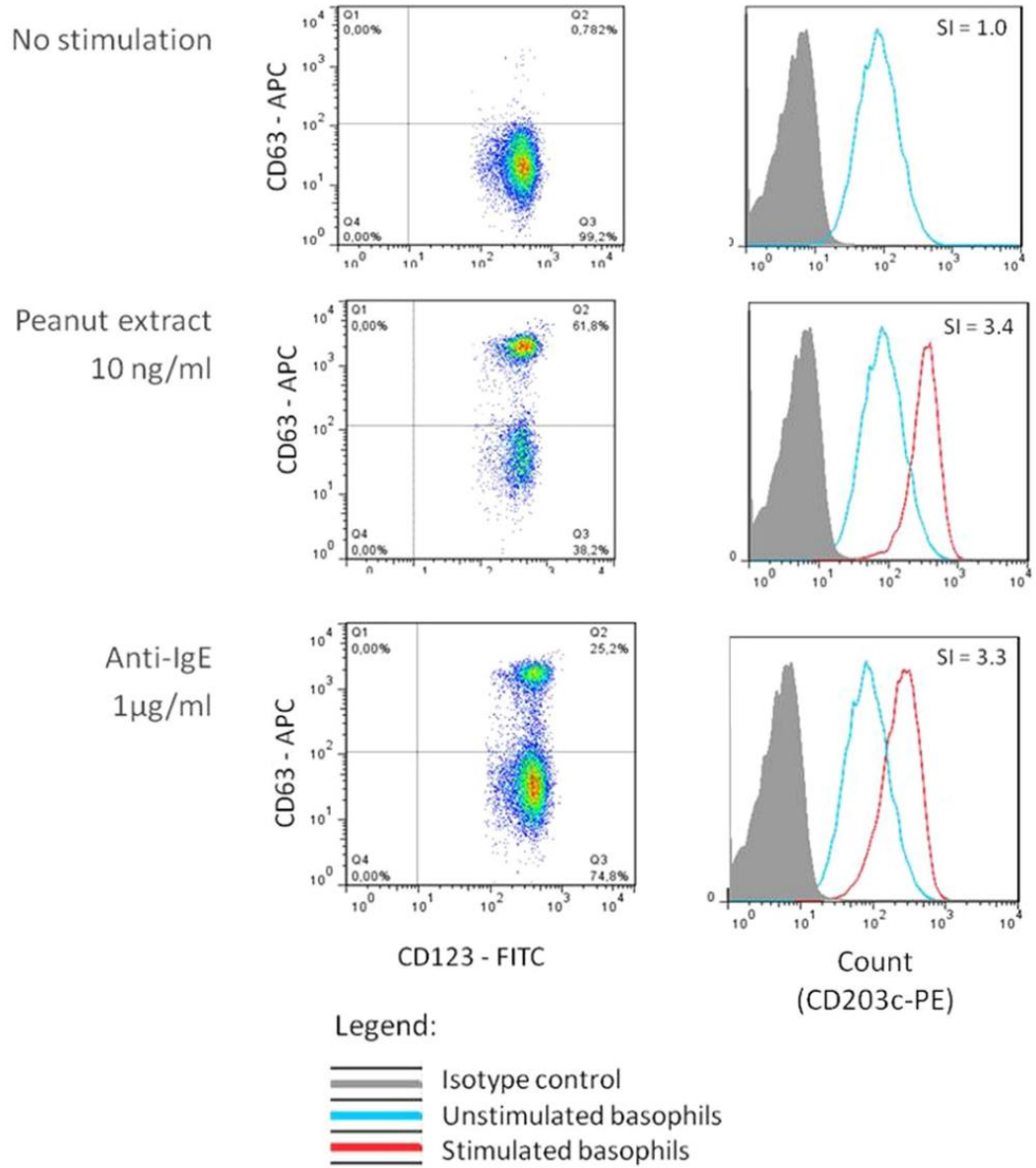
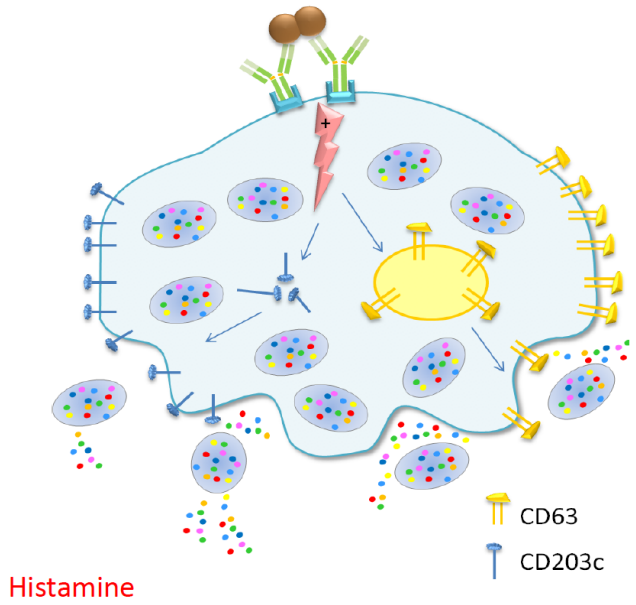
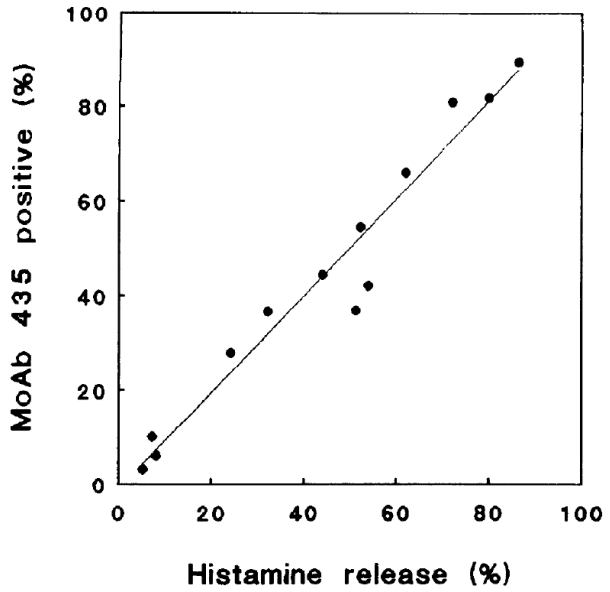


Fig. 2 *Dot plots* and histograms showing the expression of CD63 and CD203c on the surface of basophils in different conditions. Unstimulated cells (negative control) and cells stimulated with peanut or with anti-IgE (positive control) are represented. The expression of CD63 is measured as the percentage of positive basophils (*left panel*) and the expression of CD203c is measured as the stimulation index (SI), i.e. the ratio of the mean fluorescence intensity of stimulated cells and the negative control (*right panel*)

Santos and Lack *Clin Transl Allergy (2016) 6:10* DOI 10.1186/s13601-016-0098-7

CD63 expression correlates with histamine release





Knol EF et al. J Allergy Clin Immunol 1991

Classical bell-shaped dose-response curve

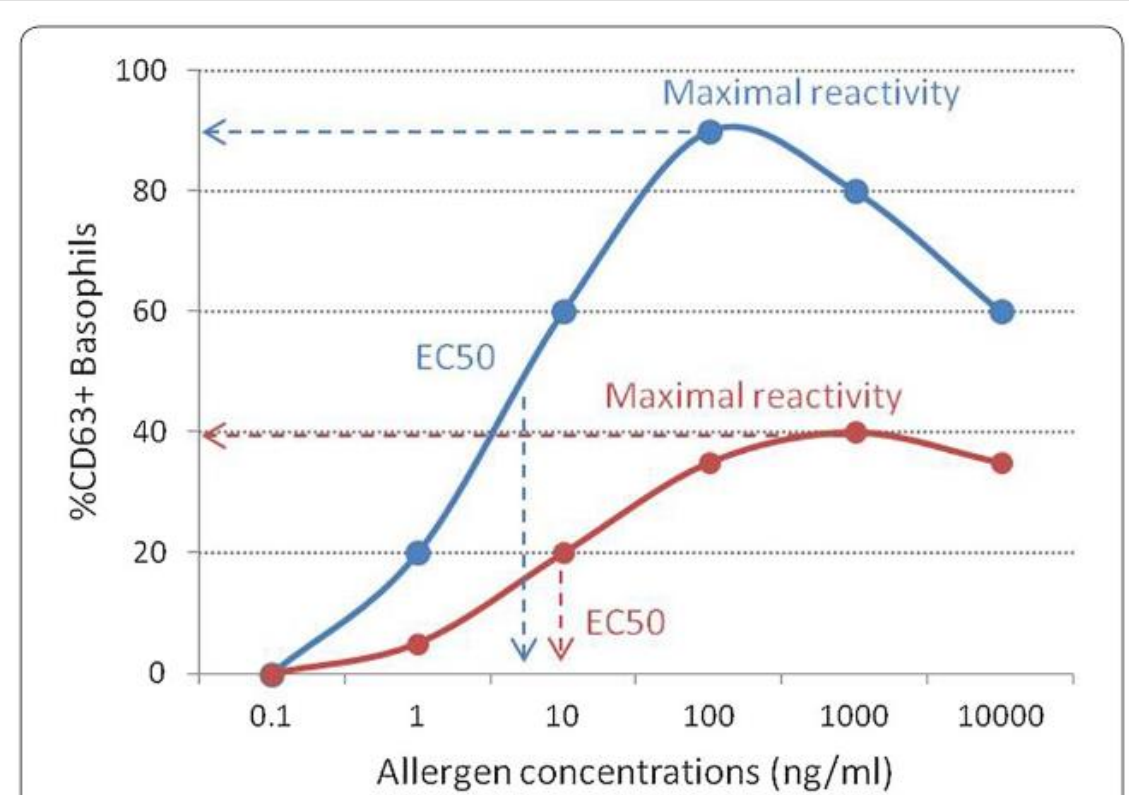


Fig. 3 Basophil reactivity and basophil sensitivity. Two examples of dose–response curves of basophil activation following stimulation with various concentrations of allergen from two different patients are represented. The proportion of CD63+ positive cells is a measure of basophil reactivity and EC_{50}, the effective concentration at 50 % of the maximal activation, is a measure of basophil sensitivity

Santos and Lack *Clin Transl Allergy (2016) 6:10* DOI 10.1186/s13601-016-0098-7

Non-responders

- It is important to document that basophils are alive and capable of mounting a response to a non-IgE stimulus, confirming that the activation test is valid
- The bacterial tripeptide, fMLP, that activates basophils through the G-protein coupled fMLP receptors, is often used as a non-IgE-mediated positive control
- After confirming that blood basophils respond to fMLP, it is important to assess whether they respond to IgE-mediated controls, such as anti-IgE
- Basophils that do not get activated in response to a stimulus through IgE, but only to non-IgE-mediated controls are designated ‘non-responders’
- Basophils of approximately 10% of the population transiently do not respond to stimulation through IgE receptor even though they express normal densities of cell surface IgE and upregulate CD63 well to an IgE-independent stimulus
- One cause of non-responsiveness is a low level of Syk phosphatase possibly in combination with elevated amounts of CD45
- More studies are needed to explore the immune mechanisms underlying non-responder phenotype and its clinical relevance

Stability of BAT

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ORIGINAL ARTICLE

CLINICAL CYTOMETRY WILEY

Validation of inducible basophil biomarkers: Time, temperature and transportation

Theodore Kim[1] | Jing Yu[2] | Henry Li[2] | Mark Scarupa[2] | Richard L. Wasserman[3] | Athena Economides[2] | Martha White[2] | Carla Ward[2] | Atul Shah[4] | Douglas Jones[5] | Melinda Rathkopf[6] | Kelly Frye[7] | Ahmet Aybar[8] | Shahrooz Shayegan[1] | Benjamin Enav[9] | Laura Ispas[10] | Denise Loizou[7] | David Fitzhugh[11] | James Tracy[12] | James Friedlander[12] | Zachary Jacobs[13] | Jonathan Matz[14] | David Golden[14] | Donald McNeil[15] | William McCann[16] | Christopher Copenhaver[16] | Jeffrey Factor[17] | Raavi Gupta[7,18] | Oral Alpan[7] | Matthew Plassmeyer[7] | Søren Ulrik Sønder[7]

It is possible to extend the stability of the basophil activation assay to 1 day for samples stored at 18–25C as well as samples shipped under ambient conditions as long as the temperature is within the 2–37C range

TABLE 2 Practical issues and considerations for optimal use of BAT in clinical trials

Practical issues	Ruggestions	Implications for clinical trials
Basophil reactivity is reduced over time.[33]	Perform BAT within a few hours (up to 24 h) of blood collection.	• Good transportation system between sites to ensure timely delivery of samples. • Test samples of all study sites within the same time frame.
Basophil reactivity can be affected by vibration and changes in temperature.[33]	Ensure method of transportation that ensure stability of temperature transfer of samples.	• Prefer transport system with temperature control for samples.
Immunosupressors, including oral corticosteroids, can reduce basophil response.[34]	Avoid performing BAT in patients who are on immunosupressors.	• Need to continue treatment with immunosupressors should be an exclusion criteria of studies using BAT.
Exposure to allergen, chronic inflammation and infection can induce basophil degranulation and homing to the tissues.[118]	Avoid performing BAT after allergen exposure or during infection or active chronic inflammatory condition.	• Blood for BAT needs to be collected prior to allergen exposure (namely challenge but not SPT). • Active infections and inflammatory conditions should be an exclusion criteria of studies using BAT.
Basophil activation can vary with the anticoagulant used.[33]	BAT can be performed in blood collected into heparin or EDTA.	Blood for BAT should be collected using the same material and methodology during studies and between sites.
Measurement of basophil activation can be influenced by the markers used to identify the basophils, by the BAT protocol and by flow cytometry.[6]	BAT should be performed with a validated method and standardized conditions.	The same reagents and protocol should be used throughout a clinical trial and flow cytometers should be standardized.
Quantification of basophil activation can vary with the method adopted for data analyses.[6,36]	Criteria should be defined for each step of flow cytometry data analyses. Automated data analyses can be considered.	The exact same methodology of analyses of flow cytometry data needs to be used between centres and throughout the clinical trial.

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REVIEW ARTICLE

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

Basophil activation test: Mechanisms and considerations for use in clinical trials and clinical practice

Alexandra F. Santos[1,2,3,4] | Oral Alpan[5] | Hans-Jürgen Hoffmann[6,7]

TABLE 1 Sensitivity and specificity of the basophil activation test to diagnose different allergic conditions

Allergic disease	Examples	Allergen stimulation	Optimal cut-off	Sensitivity	Specificity
Food allergy	Peanut allergy[2]	Peanut extract 0.1-10,000 ng/ml	8.11% $CD63^+$ basophils	98%	96%
	Egg allergy[113]	Ovalbumin 0.1–100 μg/ml	5% $CD63^+$ basophils	77%	100%
Drug allergy	Beta-lactams[114]	Various	5% $CD63^+$ basophils	55%	80%
	Neuro-muscular blocking agents[115]	Rocuronium	4% $CD63^+$ basophils	80%	96%
Insect venom allergy	Wasp venom[116]	Wasp venom, 0.0001–1 μg/ml	10% $CD63^+$ basophils	85%	83%
	Bee venom[116]	Bee venom, 0.0001–1 μg/ml	10% $CD63^+$ basophils	91%	93%
Respiratory allergy	Grass pollen[40]	Grass pollen extract, 100–0.0001 SQU/ml	2.5% $CD63^+$ basophils	ND	ND
	Aspergillus[117]	A fumigatus extract (10 μl) or rAsp f 1	ND	ND	ND

TABLE 3 Indications for the basophil activation test in the clinical setting

Indications	References
Confirmation of diagnosis	
Food allergy	Santos & Shreffler 2017[60]
Drug allergy	Aranda 2011[21]; Ebo 2006[119]
Venom allergy	Eberlein 2012[64]
Occupational allergy	Hansen 2014[120]
Allergic rhinitis	Nopp 2013[41]
Local allergic rhinitis	Campo 2015[121]
Allergic asthma	Dahlen 2011[40]
Allergic bronco-pulmonary aspergillosis	Gernez 2016[117]
Eligibility for treatment	
Allergen-specific immunotherapy	Schmid 2014[45]
Anti-IgE	Johansson 2009[90]
Other immunomodulatory treatments	
Monitoring	
Natural resolution of food allergy	Wanich 2009[66]; Berin 2008[67]
Response to allergen-specific immunotherapy	Schmid 2014/2020[45,48]
Response to anti-IgE	Nopp 2007[50]

TABLE 4 Clinical applications of the basophil activation test

Key clinical messages
• A basophil activation test above the positive cut-off supports the diagnosis of IgE-mediated allergy. • Basophil reactivity and basophil sensitivity decrease over the course of allergen-specific immunotherapy and EC50 (or CD-sens) seem to be particularly important in reflecting the change in clinical allergen threshold. • Omalizumab decreases basophil response to allergen as a consequence of the decrease in IgE receptor density and circulating IgE.

- Can BAT sufficiently help to reduce the risk and burden (time, space, labor)of OFC?
- Helps to give more evidence in difficult cases
- What else may it help with regarding food allergy?

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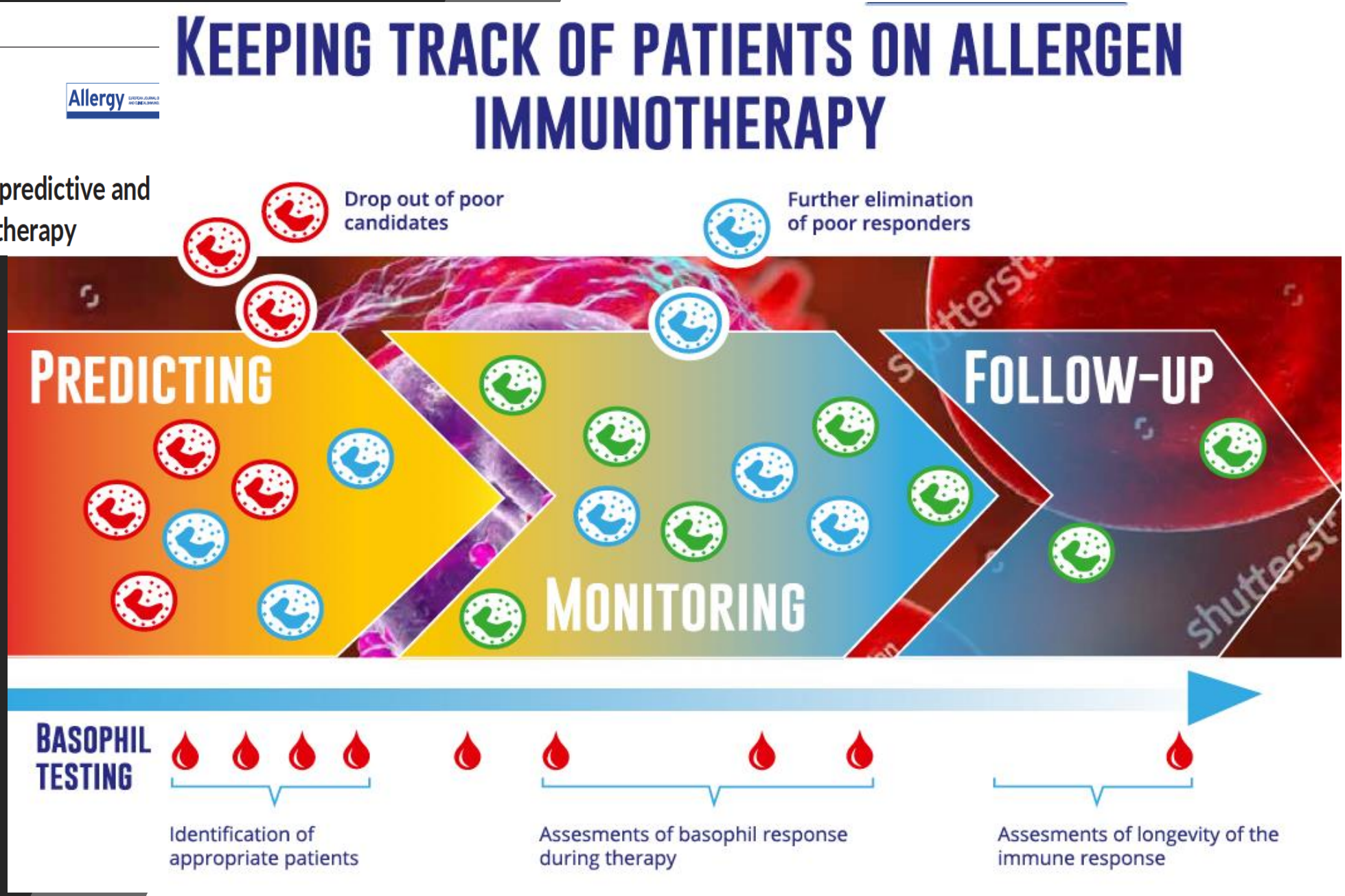
EDITORIAL

Allergy

Basophil activation test: A diagnostic, predictive and monitoring assay for allergen immunotherapy

Can BAT help monitor OIT progress?

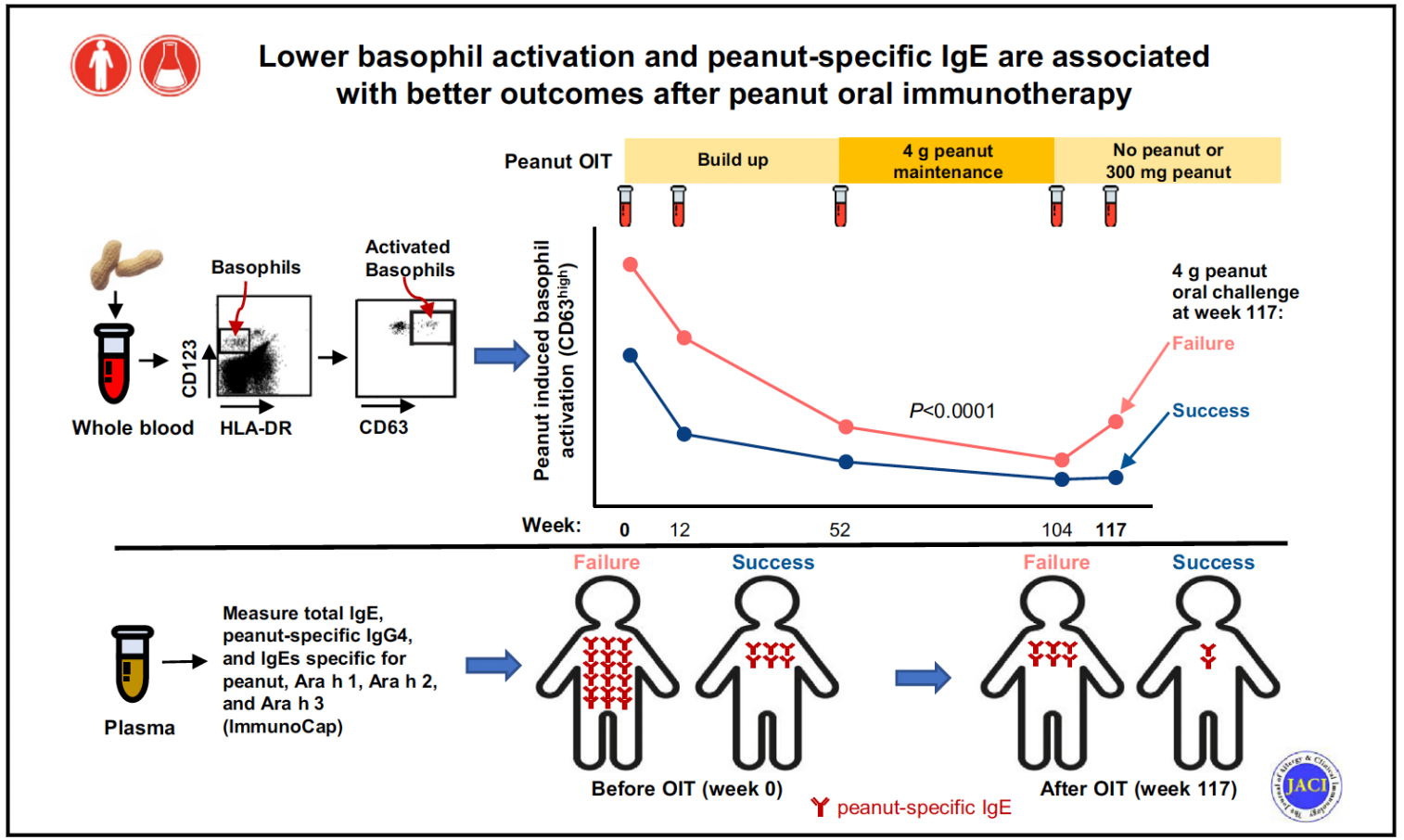


Predicting

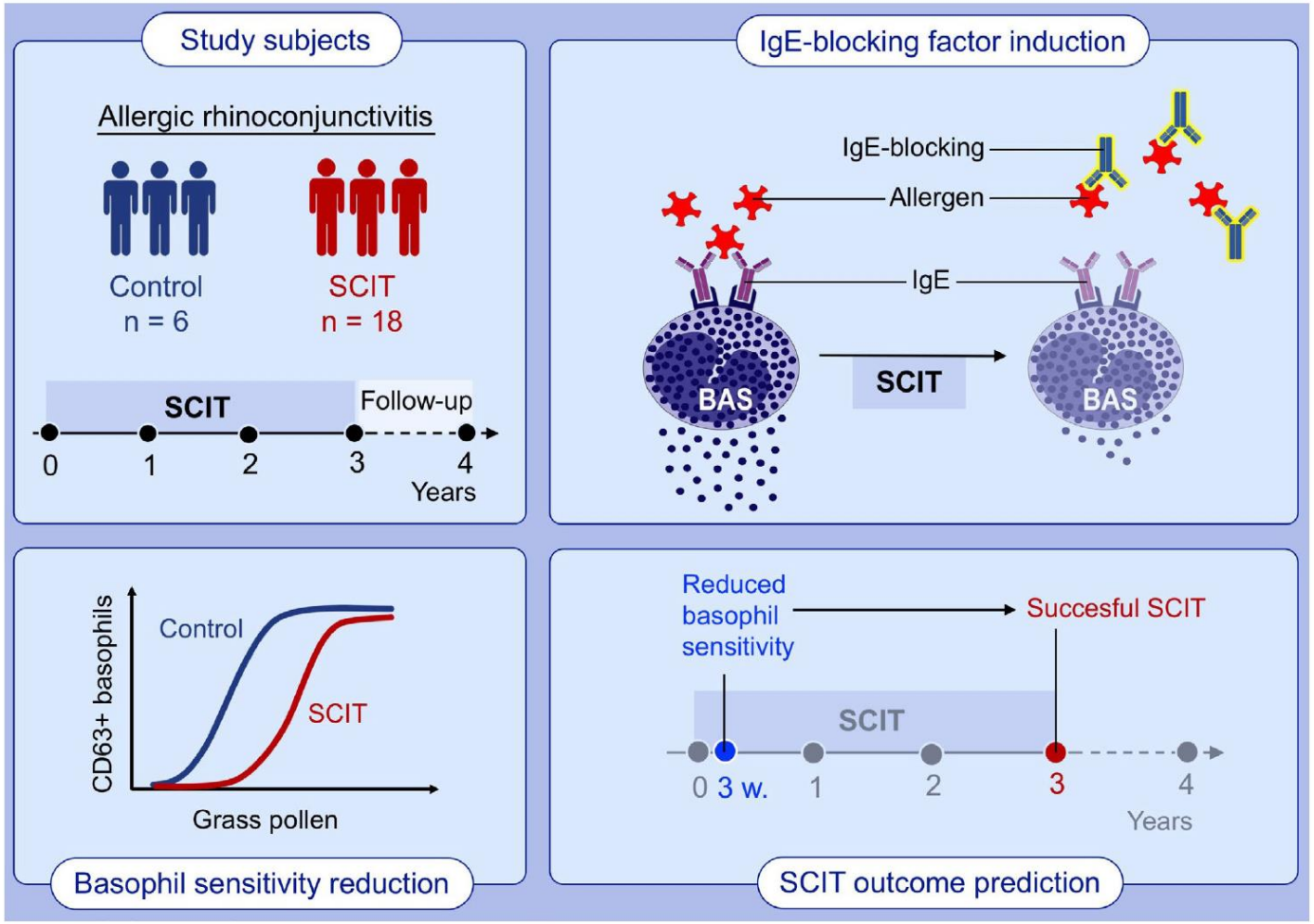
Sustained successful peanut oral immunotherapy associated with low basophil activation and peanut-specific IgE

Mindy Tsai, DMSc,[a,b]* Kaori Mukai, PhD,[a,b]* R. Sharon Chinthrajah, MD,[b,c] Kari C. Nadeau, MD, PhD,[b,c] and Stephen J. Galli, MD[a,b,d] *Stanford, Calif*

GRAPHICAL ABSTRACT



Monitoring



GRAPHICAL ABSTRACT

Grass SCIT leads to a 447-fold decrease in basophil sensitivity during the first treatment year. AUC of IgE-blocking factor correlates to nasal challenge and SPT. Decrease in basophil sensitivity during the first three weeks predicts the long-term clinical improvement during subcutaneous immunotherapy and may represent the biomarker of choice for recommending continued SCIT treatment.

FOLLOW UP:
BAT to shrimp 2 years post OIT graduation

BASOPHIL PHENOTYPING	% positive CD63	CD63 Reference Range	CD203c MFI fold change	CD203c Reference Range
CD45/CD123/CD193/IgE/CD63/CD203c	0.8		N/A	
Antigen KLH: CD63+CD203c+	0.8		0.9	
Antigen IgE: CD63+CD203c+	0.3		1.1	
Antigen fMLP: CD63+CD203c+	19.6		1.6	
Antigen 1:Shrimp				
Basophil Phenotype 10,000 ng/ml	1.0	<1.60	1.11 H	<1.1
Basophil Phenotype 1000 ng/ml	1.5	<1.60	0.93	<1.1
Basophil Phenotype 100 ng/ml	1.0	<1.60	0.90	<1.1
Basophil Phenotype 10 ng/ml	0.8	<1.60	0.99	<1.1
Basophil Phenotype 1 ng/ml	2.0 H	<1.60	1.03	<1.1
Basophil Phenotype 0.1 ng/ml	0.8	<1.60	1.06	<1.1

FOLLOW UP:
BAT to peanut 2 years post OIT

LYMPHOCYTE AND BASOPHIL PHENOTYPE RESULTS

% basophils of WBC

No reference labs available

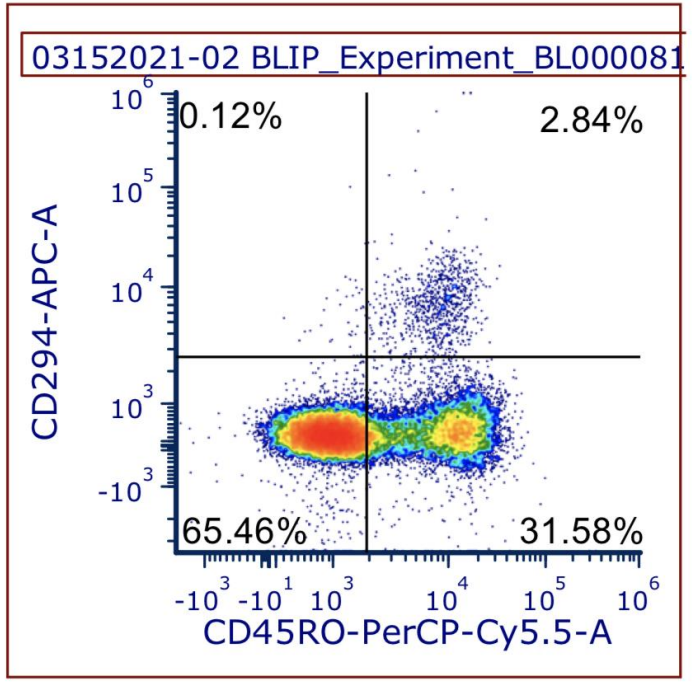
CD45+/IgE+	1.19
CD45+/CD123+/CD193+	0.64

BASOPHIL PHENOTYPING	**% positive CD63**	**CD63 Reference Range**	**CD203c MFI fold change**		**CD203c Reference Range**
CD45/CD123/CD193/IgE/CD63/CD203c	0.1		N/A		
Antigen KLH: CD63+CD203c+	1.0		1.2		
Antigen IgE: CD63+CD203c+	0.5		0.9		
Antigen fMLP: CD63+CD203c+	40.2		6.2		
Antigen 1:Peanut					
Basophil Phenotype 10,000 ng/ml	0.1	<1	1.11	H	<1.1
Basophil Phenotype 1000 ng/ml	0.5	<1	1.27	H	<1.1
Basophil Phenotype 100 ng/ml	0.3	<1	1.30	H	<1.1
Basophil Phenotype 10 ng/ml	0.8	<1	1.07		<1.1
Basophil Phenotype 1 ng/ml	0.0	<1	1.20	H	<1.1
Basophil Phenotype 0.1 ng/ml	0.0	<1	0.98		<1.1

Clinical hx allergy, IgE's negative. Failed past OFC in the past. Saw me for a second opinion

BASOPHIL PHENOTYPING	% positive CD63		CD63 Reference Range	CD203c MFI fold change		CD203c Reference Range
CD45/CD123/CD193/IgE/CD63/CD203c	1.2			N/A		
Antigen KLH: CD63+CD203c+	1.2			1.0		
Antigen IgE: CD63+CD203c+	15.4			3.8		
Antigen fMLP: CD63+CD203c+	47.1			3.4		
Antigen 1:CASHEW						
Basophil Phenotype 10,000 ng/ml	1.4		<2.40	1.02		<1.1
Basophil Phenotype 1000 ng/ml	4.1	H	<2.40	1.35	H	<1.1
Basophil Phenotype 100 ng/ml	3.6	H	<2.40	1.56	H	<1.1
Basophil Phenotype 10 ng/ml	4.0	H	<2.40	1.41	H	<1.1
Basophil Phenotype 1 ng/ml	3.9	H	<2.40	1.04		<1.1
Basophil Phenotype 0.1 ng/ml	0.6		<2.40	0.85		<1.1
Antigen 2: PEANUT						
Basophil Phenotype 10,000 ng/ml	0.9		<2.40	1.02		<1.1
Basophil Phenotype 1000 ng/ml	7.9	H	<2.40	1.75	H	<1.1
Basophil Phenotype 100 ng/ml	6.2	H	<2.40	1.66	H	<1.1
Basophil Phenotype 10 ng/ml	4.8	H	<2.40	1.49	H	<1.1
Basophil Phenotype 1 ng/ml	1.3		<2.40	1.24	H	<1.1
Basophil Phenotype 0.1 ng/ml	0.3		<2.40	0.90		<1.1

Was going to start OIT to milk and egg. BAT is classic, but increased CHTR2



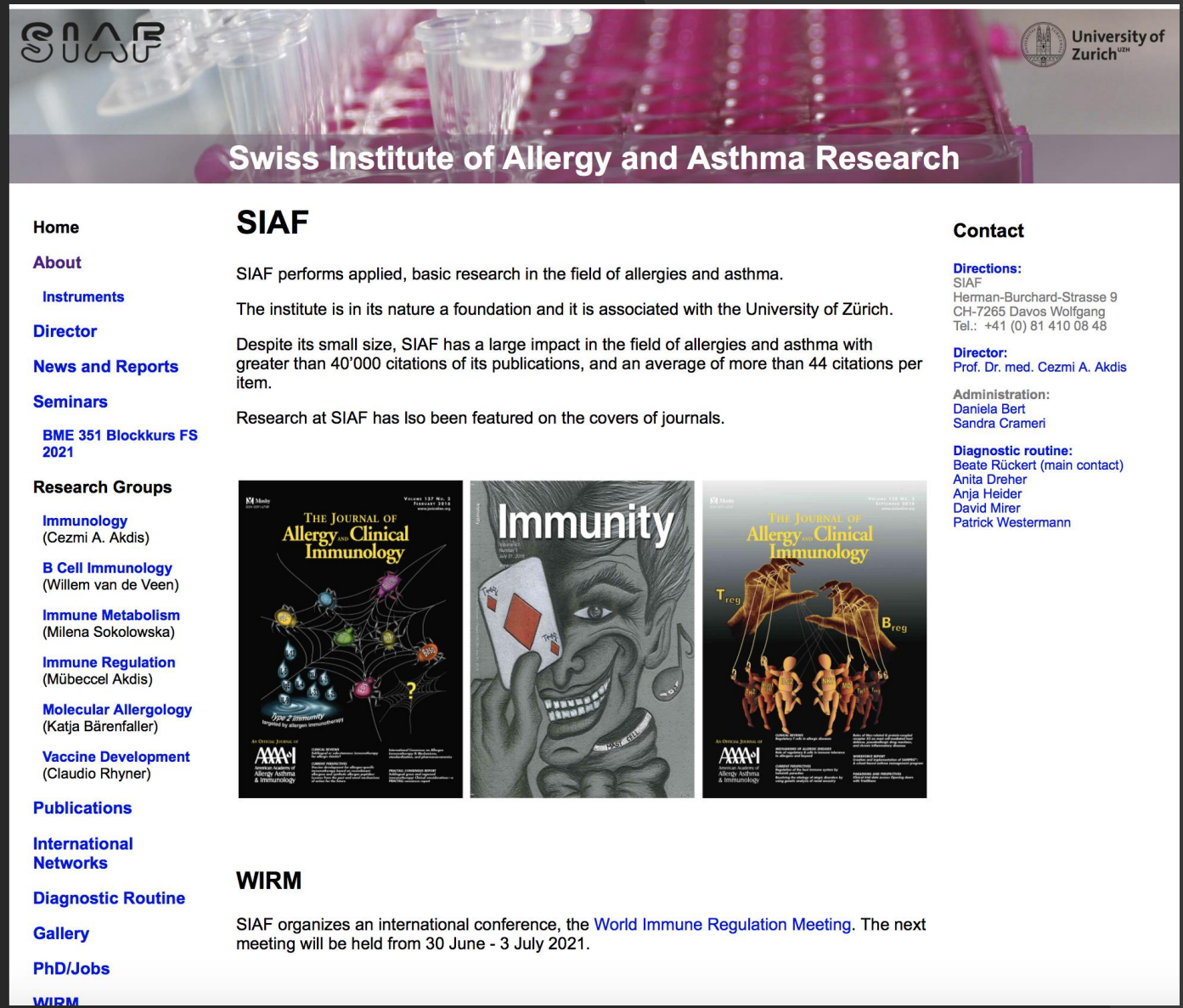
LYMPHOCYTE AND BASOPHIL PHENOTYPE RESULTS

	% of	% positive	Reference range
YMPHOCYTE PHENOTYPING			
D3	Lymphocytes	83.6	54.5 - 88.4
D20	Lymphocytes	12.4	8.9 - 33.6
D16+ or CD56+/CD3-	Lymphocytes	14.9	1.1 - 15.4
BNK	Lymphocytes	110.9	
D3/CD4	CD3	50.1	44.2 - 77.5
D3/CD8	CD3	35.1	15.2 - 52
D3/CD4/CRTH2	CD3/CD4	3.0	0.2 - 3.3
D3/CD4/CD45RO-/CRTH2	CD3/CD4/CD45RO-	0.2	0.07 - 3
D3/CD4/CD45RO/CRTH2	CD3/CD4/CD45RO	8.3	
D3/CD8/CRTH2	CD3/CD8	0.4	<2.43
D3/CD8/CD45RO-/CRTH2	CD3/CD8/CD45RO-	0.3	
D3/CD8/CD45RO/CRTH2	CD3/CD8/CD45RO	1.2	<1.45

BASOPHIL PHENOTYPING	**% positive CD63**	**CD63 Reference Range**	**CD203c MFI fold change**	**CD203c Reference Range**
CD45/CD123/CD193/IgE/CD63/CD203c	1.2		N/A	
Antigen KLH: CD63+CD203c+	1.2		1.0	
Antigen IgE: CD63+CD203c+	15.4		3.8	
Antigen fMLP: CD63+CD203c+	47.1		3.4	
Antigen 1:CASHEW				
Basophil Phenotype 10,000 ng/ml	1.4	<2.40	1.02	<1.1
Basophil Phenotype 1000 ng/ml	4.1 H	<2.40	1.35 H	<1.1
Basophil Phenotype 100 ng/ml	3.6 H	<2.40	1.56 H	<1.1
Basophil Phenotype 10 ng/ml	4.0 H	<2.40	1.41 H	<1.1
Basophil Phenotype 1 ng/ml	3.9 H	<2.40	1.04	<1.1
Basophil Phenotype 0.1 ng/ml	0.6	<2.40	0.85	<1.1
Antigen 2: PEANUT				
Basophil Phenotype 10,000 ng/ml	0.9	<2.40	1.02	<1.1
Basophil Phenotype 1000 ng/ml	7.9 H	<2.40	1.75 H	<1.1
Basophil Phenotype 100 ng/ml	6.2 H	<2.40	1.66 H	<1.1
Basophil Phenotype 10 ng/ml	4.8 H	<2.40	1.49 H	<1.1
Basophil Phenotype 1 ng/ml	1.3	<2.40	1.24 H	<1.1
Basophil Phenotype 0.1 ng/ml	0.3	<2.40	0.90	<1.1

Current questions & future of BAT

- How non-reactives correlate with clinical outcomes?
- Correlation of different dose response curves
- OIT data is scarce
  - Can Pre-OIT values or tests 3 weeks into OIT predict outcomes? Can it help with protocol adjustments?
  - Can it help determine appropriate dosing? (e.g. Low dose vs high dose?)
  - Is CRTH2 another marker that can be used?
  - Sustained unresponsiveness?



AmeriBAT collaboration 2021:

- Amerimmune; Fairfax, **VA**
- Rocky Mountain Allergy at Tanner Clinic; Layton, **UT**
- New York Allergy Immunology and Research Lab, Centereach, **NY**
- Institute for Asthma and Allergy, Wheaton, **MD**
- Allergy Asthma and Immunology Relief of Charlotte, Charlotte, **NC**
- Oklahoma Institute of Allergy Asthma and Immunology, Oklahoma City, **OK**
- Optimed, Columbus, **OH**