ONLINE REPOSITORY

MATERIALS AND METHODS

Reagents

For *in vitro* experiments on murine humanized bone marrow–derived mast cells (MCs), chimeric humanized anti-NP IgE (cIgE) (MCA333S clone JW8/1 Serotec BioRad, Hercules, Calif) and its hapten-specific antigen NP-HSA/bovine serum albumin (BSA) (N-50051 and N-5050L; Biosearch Technologies, Petaluma, Calif) were used at different concentrations (0.5 μg and 0.01-100 ng, respectively). Human serum (up to 10%) from an allergic donor containing IgE against methotrexate (in 200 μL) followed by α-hIgE challenge (Goat anti-Human IgE-Affinity Purified; Immunology Consultants Laboratory, Inc., Portland, Ore) was assessed to find the antigen target dose (0.5 μg, experiment not shown). To optimize the system, MCs were also sensitized using different percentages of patients' serum and then challenged with the same antigen doses to find the optimal serum dose (5%) to sensitize the cells (the dose that produces the higher β-hexosaminidase release during challenge). BSA (10 ng) or Razin Medium was used as a control.

Cells and culture conditions

For the generation of MCs, femurs of hFcεRIα^{+}/mFcεRIα$^{-/-}$ and mFcεRIα$^{-/-}$ (MuKO) mice were flushed and progenitor cells were cultured as described[E1] in RPMI medium supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, Mass), 10,000 U/mL Pen-Strep (Gibco), 2 mM L-glutamine, 50 μM β-mercaptoethanol (Gibco), 10 ng/mL recombinant murine IL-3, and stem cell factor (PeproTech, Rocky Hill, NJ).

FcεRI cross-linking, sFcεRI production, and β-hexosaminidase release assay

MCs (1×10^6 cells in 1 mL per condition) cultured in Razin Medium were loaded overnight with cIgE (0.5 μg/mL) or with medium (in 200 μL) containing up to 10% serum from patients who were allergic to methotrexate. MCs were stimulated either with single or sequential doses of 4-hydroxy-3-nitrophenylacetyl bovine serum albumin (NP-BSA) (0.01-100 ng/mL) or anti-hIgE (0.5 μg) for 10 or 110 minutes. Desensitization (DS) was performed as previously described[E2,E3] and is outlined in Figure 2.

Culture supernatants were collected and analyzed by the enzyme-linked immunosorbent assay for sFcεRI and β-hexosaminidase levels as previously described.[E2,E4,E5]

In vitro DS on humanized bone marrow–derived MCs

MCs and MuKO cells were harvested and cultured as described, and activation or DS was performed. *In vitro* DS protocols were used as previously described.[E2,E3] Briefly, cells were loaded overnight with cIgE (0.5 μg/mL) or with medium (in 200 μL) containing up to 10% serum from a patient allergic to methotrexate. Cells were stimulated either with single or sequential doses of NP-BSA (0.01-100 ng/mL) or anti-hIgE (0.5 μg) for 10 or 110 minutes, as outlined in Figure 2, *A*, and Figure E3 (available in this article's Online Repository at www.jaci-inpractice.org).

Mice

Mice were all housed in specific pathogen-free conditions according to the National Institutes of Health, and all experiments were performed with cohoused and littermate controlled cohorts that contained both genders. All animal studies were approved by the Boston Children's Hospital Institutional Animal Care and Use Committee.

Patient information

Patients with cancer who experienced a hypersensitivity reaction to carboplatin or oxaliplatin were recruited, and serum was collected for sFcεRI, IgE, and tryptase detection. Allergic patients were defined by >3 mm wheal size in skin test (ST)[E6-E9] with the culprit drug and any hypersensitivity IgE-mediated/mast cell activation–related symptoms[E6,E8-E14] during platin infusion (first reaction) from cutaneous to even anaphylaxis. The initial reaction was graded following Brown's classification in: mild/grade 1 (1 symptomatic organ system), moderate/grade 2 (2 or more systems involved), and severe/grade 3 (more than 2 systems affected and/or vital sign changes). The presence of other adverse drug reactions was reported by the patients.

Serum used in humanized bone-marrow derived mast cell sensitization was collected from a 70-year-old man with lymphoma. He presented with an immediate reaction during the 15th life time exposure to methotrexate with positive ST.

Control atopic patients were recruited and serum was collected for sFcεRI and IgE detection. Inclusion criteria were no history of cancer and allergen-specific IgE levels >0.35 kU_A/L to 1 or more allergens.

A total of 14 patients with cancer and 12 atopic controls were used in the analysis. All patients included in the study gave written informed consent and were recruited after approved application from the Research Consent Form for Biomedical Research-Dana-Farber/Harvard Cancer Center-BIDMC/BCH/BWH/DFCI/MGH/Partners Network Affiliates (DFCI Protocol Number: 13-288) or the Ethics Commission, Medical University of Vienna (EK Nr: 079/2009). Patients were followed up and their clinical history during further DS protocols was analyzed.

Serum sample collection

Blood samples were collected on the day of DS before and at the end of the procedure. A yellow cap BD Vacutainer SST II Advance tube was used to obtain and separate the serum sample (the tubes contain silica to activate clotting of the specimen and a gel that forms a barrier between the clot and the serum after centrifugation). After forming a clot (30 minutes), tubes were centrifuged at 1300 to 2000 *g* for 10 minutes at 25°C. Next, serum was collected and stored at −80°C. Serum samples from controls were collected and processed as described.

Drug DS in patients with cancer

Patients with cancer allergic to oxaliplatin or carboplatin entered a drug DS protocol, as previously described and shown in Figure E3. DS protocols with 16 to 12 steps, escalating the dose 2 to 2.5 times every 15 minutes, were applied.[E6,E8-E14] The initial concentration of the solution in a 4 bags/16 steps protocol was 1/1000, and 1/100 was the first bag concentration in a 3

bags/12 steps protocol, reaching the target dose at the end of the procedure.[E6] All patients received standard premedication. Omalizumab (anti-IgE therapy) was used in patient 1, who was highly sensitized and experienced severe reactions during previous DS (Table E1). At the time of serum collection, patients 1 and 6 experienced an IgE-mediated reaction grade 1 during DS. During further DS, patients 1, 6, and 8 experienced IgE-mediated reactions with different grades at the referred DS number (Table E2). Patient 14 experienced a cytokine release reaction grade 2 at DS 13, 14, 15, and 16. All DS were completed until the patients received the treatment target dose for their oncologic disease, or until the patient deceased, changed treatment, or other reason (Table E2).

Statistical analyses

All statistical analyses were performed using Prism 7 (GraphPad Software), and results are shown as mean ± standard error of the mean of the indicated number of individual data points or independent experiments. Correlations were calculated by the Pearson rank correlation test and correlation coefficients are displayed as "*r*." Statistical analysis was performed using an unpaired or a paired *t*-test for comparison between 2 groups, a 1-way analysis of variance (ANOVA) test plus Tukey's multiple correction for more than 2 unmatched groups and a 2-way ANOVA test plus Tukey's multiple correction for multiple comparisons between 3 or more groups. A P value $\leq .05$ was considered significant.

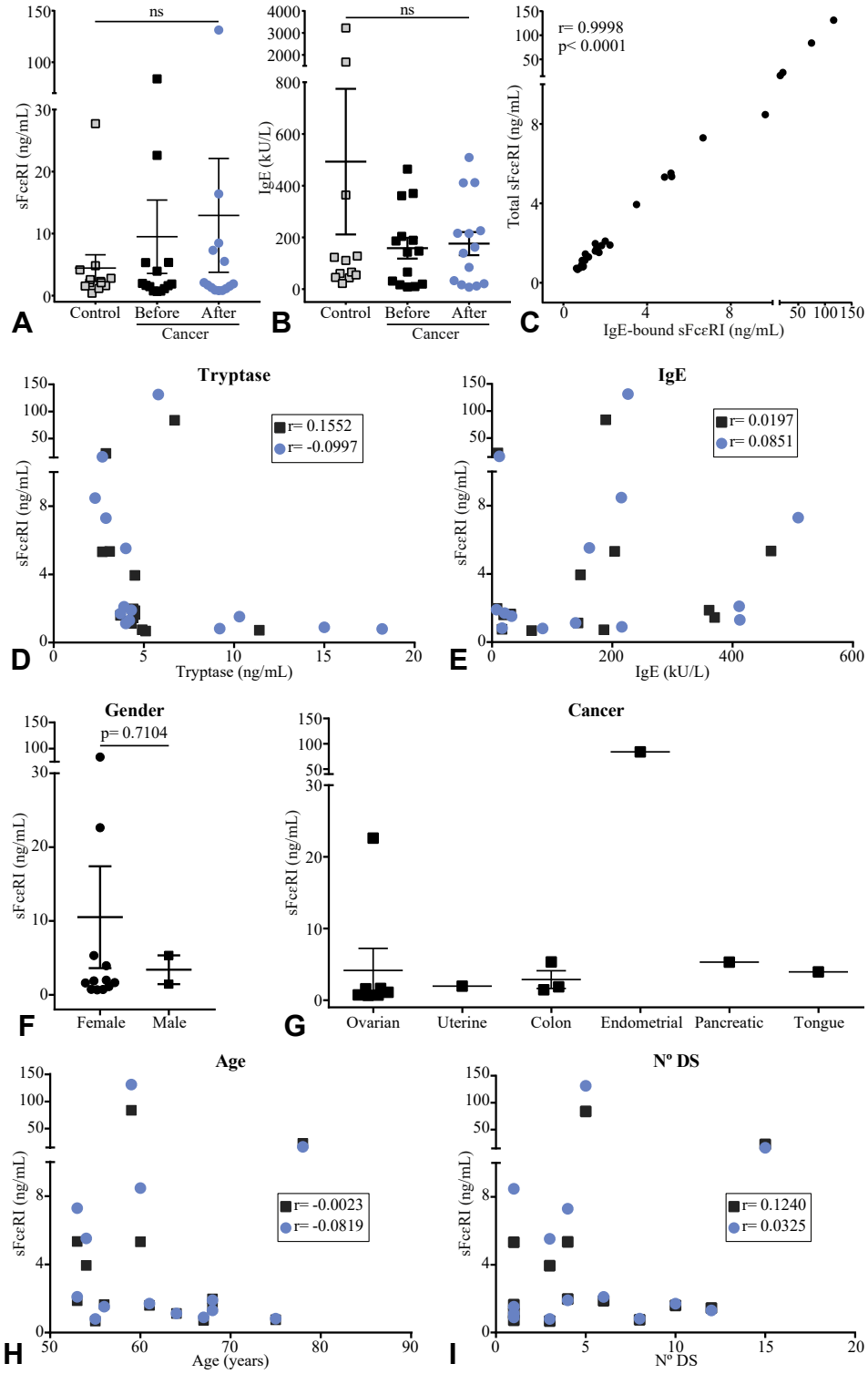


FIGURE E1. Levels of sFcεRI in controls and drug-allergic patients with cancer. Comparison of sFcεRI or IgE levels in control and patients with cancer (**A**, **B**). Correlation between total and IgE-bound sFcεRI levels (**C**), tryptase levels (**D**), total IgE levels (**E**), gender (**F**), cancer type (**G**), age (**H**), and number of DS (**I**) in patients with cancer before (black squares) and after (blue circles) DS. An unpaired *t*-test or a 1-way ANOVA test plus Tukey's multiple correction, or Pearson *r* correlation analysis was performed. *ANOVA*, Analysis of variance; *DS*, desensitization; *ns*, not significant; *sFcεRI*, soluble FcεRI.

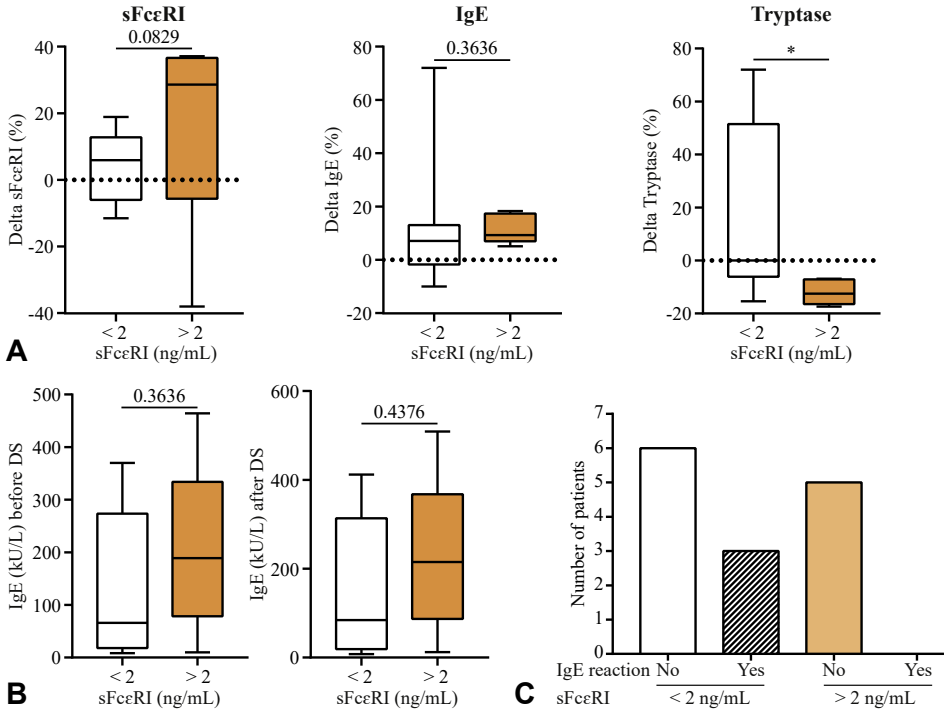
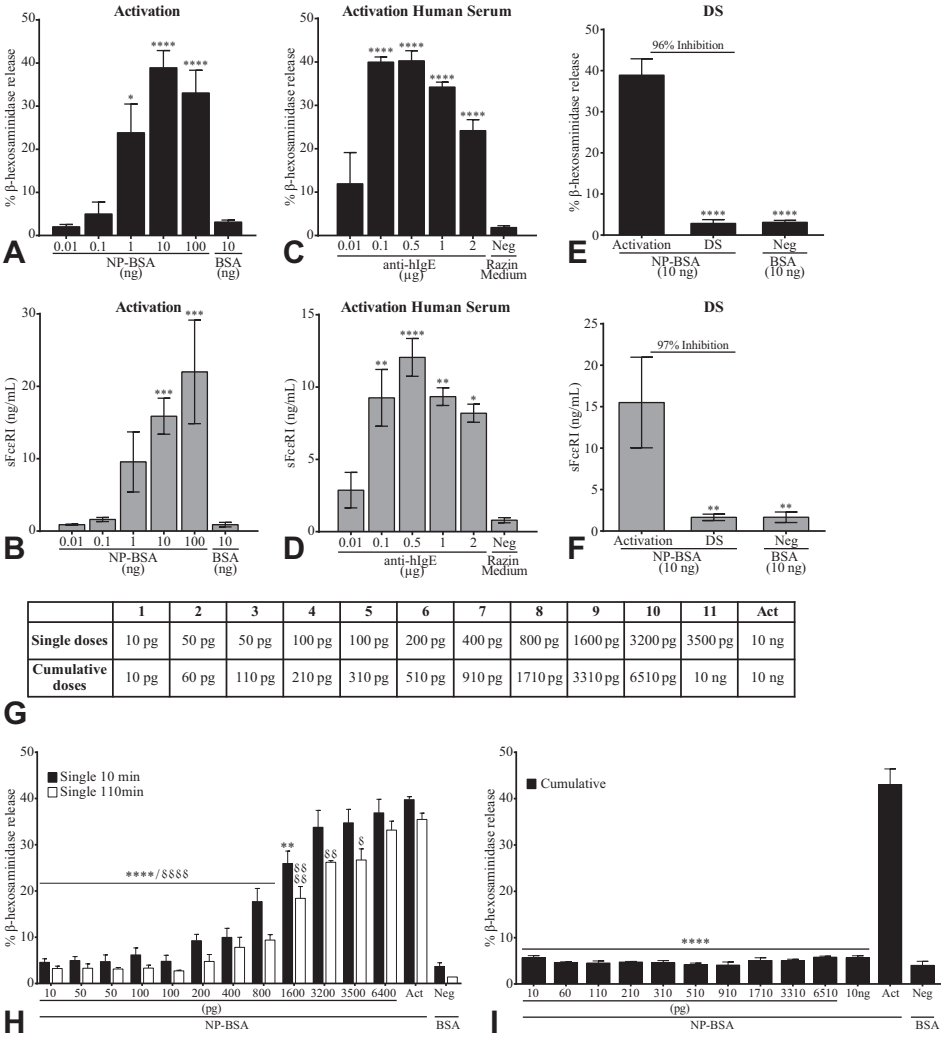


FIGURE E2. Modulation of sFcεRI, IgE, and tryptase levels by DS. Difference of sFcεRI, IgE, and tryptase levels between before and after DS represented as percentage (**A**). Total IgE levels before and after DS (**B**). Patients (n = 5) with sFcεRI levels >2 ng/mL (white bar) and patients (n = 9) with sFcεRI levels <2 ng/mL (orange bar) are represented as box and whisker graphs (minimum to maximum). Number of patients to suffer an IgE-mediated reaction during further DS (**C**). A Mann-Whitney test was performed, where $*P < .05$. *DS*, Desensitization; *sFcεRI*, soluble FcεRI.



	1	2	3	4	5	6	7	8	9	10	11	Act
Single doses	10 pg	50 pg	50 pg	100 pg	100 pg	200 pg	400 pg	800 pg	1600 pg	3200 pg	3500 pg	10 ng
Cumulative doses	10 pg	60 pg	110 pg	210 pg	310 pg	510 pg	910 pg	1710 pg	3310 pg	6510 pg	10 ng	10 ng

FIGURE E3. Activation of MCs triggers release of sFcεRI that is inhibited by cumulative doses. A total of 1×10^6/mL MCs were loaded overnight with 0.5 µg/mL anti-NP cIgE (**A**, **B**) or up to 10% allergic human serum in 200 µL (**C**, **D**) followed by a single challenge with 100-0.1 ng/mL NP-BSA (**A**, **B**) or 2-0.01 µg/mL anti-hIgE (**C**, **D**). A total of 1×10^6/mL MCs or MuKO (mFcεRIα$^{-/-}$) cells were loaded overnight with 0.5 µg/mL anti-NP cIgE (**E**, **F**) followed by DS with 10 ng/mL NP-BSA. Percentage of β-hexosaminidase release (**A**, **C**, **E**) and total sFcεRI levels (**B**, **D**, **F**) were measured. Layout of antigen doses for single and cumulative experiments (**G**). A total of 1×10^6/mL MCs were loaded overnight with 0.5 µg/mL anti-NP cIgE (**H**, **I**) followed by a single-dose challenge or cumulative doses (10 pg/mL to 10 ng/mL NP-BSA) for 10 or 110 minutes. A single challenge with 10 ng/mL NP-BSA (Act), 10 ng/mL BSA (Neg), or Razin Medium was used as a control. Data represent mean ± SEM of n = 3-8 independent experiments. A 1-way ANOVA test plus Tukey's multiple correction was performed, where */δ$P < .05$, **/δδ$P < .01$, ***$P < .001$, and ****/δδδδ$P < .0001$ compared with controls. *Represents statistics between conditions among black bars (**H**) and cumulative (**I**) data sets. δRepresents statistics between conditions among white bars (**H**). *ANOVA*, Analysis of variance; *DS*, desensitization; *MC*, mast cell; *nd*, not detected; *NP-BSA*, 4-hydroxy-3-nitrophenylacetyl bovine serum albumin; *SEM*, standard error of the mean; *sFcεRI*, soluble FcεRI.

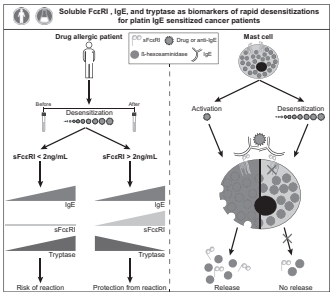


FIGURE E4. Soluble FcεRI can be used as a biomarker during *in vivo* desensitization protocols to identify patients at risk or under protection from reactions, together with serum IgE and tryptase levels. *In vitro* desensitization protocols on mast cells showed that β-hexosaminidase and soluble FcεRI release were inhibited on cumulative doses of antigen. *sFcεRI*, Soluble FcεRI.

TABLE E1. Clinical and serological characteristics of patients with cancer

												sFcεRI (ng/mL)			IgE (kU/L)			Tryptase (ng/mL)		
Patient	Age (y)	Gender	IgE disease	ADR	Cancer type	Drug	Infusion at which reaction occurred	Grade of 1st reaction	ST	No. of DS	Reaction during DS	Before	After	Delta	Before	After	Delta	Before	After	Delta
1	55	F	−	Yes	Ovarian	C	8	2	+	3	Y (1)*	0.68	0.80	15%	66.00	84.20	21.6%	5.10	18.20†	72%
2	75	F	AA+AR	Yes	Ovarian	C	12	1	+	8	−	0.75	0.83	9.6%	16.80	16.70	−0.6%	4.90	9.20†	46.7%
3	68	F	AR	Yes	Uterine	C	16	3	+	4	−	1.98	1.90	−4.2%	8.33	7.57	−10%	4.40	4.30	−2.3%
4	64	F	−	−	Ovarian	C	7	2	+	1	−	1.13	1.13	0%	143.00	139.00	−2.9%	4.30	4.00	−7.5%
5	61	F	−	Yes	Ovarian	C	9	1	+	10	−	1.60	1.70	5.9%	19.50	21.00	7.1%	3.70	3.70	0%
6	67	F	−	Yes	Ovarian	C	9	3	+	1	Y (1)*	0.73	0.90	18.9%	186.00	216.00	13.9%	11.40	15.00†	24%
7	53	F	−	Yes	Colon	O	10	2	+	6	−	1.88	2.10	10.5%	361.00	411.00	12.2%	4.50	3.90	−15.4%
8	56	F	CD	−	Ovarian	C	13	2	+	1	−	1.65	1.53	−7.8%	31.20	32.60	4.3%	4.50	10.30†	56.3%
9‡	78	F	AC+CD	−	Ovarian	C	28	1	+	15	−	22.63	16.40	−38%	9.88	12.10	18.3%	2.90	2.70	−7.4%
10‡	59	F	−	−	Endometrial	C	9	3	+	5	−	83.90	131.25	36.1%	189.00	226.00	16.4%	6.70	5.80	−15.5%
11‡	53	M	−	−	Colon	O	7	1	+	4	−	5.35	7.30	26.7%	464.00	509.00	8.8%	3.10	2.90	−6.9%
12	68	M	−	−	Colon	O	12	3	+	12	−	1.45	1.30	−11.5%	370.00	412.00	10.2%	4.40	4.20	−4.8%
13‡	60	F	FA	Yes	Pancreatic	O	7	2	+	1	−	5.33	8.47	37.1%	204.00	215.00	5.1%	2.70	2.30	−17.4%
14‡	54	F	AR	−	Tongue	C	10	3	+	3	−	3.94	5.52	28.6%	147.00	162.00	9.3%	4.50	4.00	−12.5%
Mean ↑														19.6%			11.6%			49.8%
Mean												9.50	12.94	9.1%	158.3	176.0	8.1%	4.8	3.8	7.8%
SD												21.30	33.1	19.9%	144.4	160.9	8.3%	2.1	0.9	28.5%

AA, Allergic asthma; *AC*, allergic conjunctivitis; *ADR*, adverse drug reaction; *AR*, allergic rhinitis; *C*, carboplatin; *CD*, contact dermatitis; *DS*, desensitization; *FA*, food allergy; *O*, oxaliplatin; *SD*, standard deviation; *sFcεRI*, soluble FcεRI; *ST*, skin test.

*Patients 1 and 6 experienced a mild (grade 1) reaction during DS. Patient 1 had received omalizumab as DS premedication. Values of total sFcεRI, total IgE, and tryptase from serum samples taken before and after DS.

†Tryptase levels greater than 11.5 ng/mL or greater than 1.2 times baseline + 2 ng/mL are considered elevated.

‡Patients with sFcεRI above 2 ng/mL.

TABLE E2. Clinical and serological characteristics of control group

Patient	Age (y)	Gender	Disease	Relevant allergen	sFcεRI (ng/mL)	IgE (kU/L)
C1	9	M	Atopy	–	0.40	43.80
C2	13	M	Allergic rhinoconjunctivitis	Grass	4.15	3225.00
C3	17	M	Diabetes mellitus	–	27.73	123.00
C4	11	M	Atopy	HDM	2.80	1673.00
C5	4	F	Bronchitis	–	2.08	65.60
C6	9	M	Atopy	–	2.30	111.00
C7	20	M	Diabetes mellitus	–	4.83	60.50
C8	8	M	Obstructive bronchitis	–	2.58	364.00
C9	16	F	Allergic asthma	Cat	1.55	45.40
C10	15	M	Diabetes mellitus	–	1.60	21.80
C11	17	M	Diabetes mellitus	–	1.15	128.00
C12	14	F	Diabetes mellitus	–	2.08	54.80
Mean					4.44	493.0
SD					7.12	934.3

Atopy was described as allergen-specific IgE sensitization to 1 or more allergen without clear allergic symptoms.
HDM, House dust mite; *SD*, standard deviation; *sFcεRI*, soluble FcεRI.

TABLE E3. Clinical characteristics of follow-up on cancer cohort

Patient	DS at consent	Total no. of DS	Reaction during further DS	No. of reactions	No. of DS	Biomarker (ng/mL)		Reaction phenotype/ grade of reaction	Step of DS	Reason stop of DS	Cancer status
						Tryptase	IL-6				
1	3	8	Y	6	1	27.30		Type I/Grade 2	12, 16	Change of treatment	Ongoing
					2			Type I/Grade 3 (not finished)	11, 16		
					3	6.90		Type I/Grade 1	11, 15		
					4			Type I/Grade 1	12, 15		
					6	6.00		Type I/Grade 1	12, 16		
					7			Type I/Grade 1	12		
2	8	14	N							Progression of disease	Deceased
3	4	7	N							Side effects from drugs	Deceased
4	1	11	N							Change of treatment	Deceased
5	10	11	N							Change of treatment	Unclear
6	1	12	Y	9	1			Type I/Grade 1	16	Change of treatment	Deceased
					2			Type I/Grade 1	12		
					3			Delayed			
					4			Delayed			
					5			Delayed			
					6			Delayed			
					7			Delayed			
					8			Delayed			
					9			Delayed			
7	6	8	N							Change of treatment	Deceased
8	1	6	Y	1	2			Type I/Grade 1	12	Change of treatment	Deceased
9*	15	20	N							Change of treatment	Ongoing
10*	5	7	N							Ongoing	Ongoing
11*	4	12	N							Clinical trial	Deceased
12	12	16	N							Change of treatment	?
13*	1	9	N							Changed due to intolerance neuropathy	Ongoing
14*	3	21	Y	4	13	3.20	36.00	CRR/Grade 2	12	Change of treatment	Deceased
					14			CRR/Grade 3	12		
					15		13.90	CRR/Grade 1	12		
					16		4.20	CRR/Grade 1	12		

?, Unknown; *CRR*, cytokine release reaction; *DS*, desensitization; *N*, no; *Y*, yes.
*Patients with soluble FcεRI titers above 2 ng/mL.

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