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Soluble FcεRI, IgE, and tryptase as potential biomarkers of rapid desensitizations for platin IgE sensitized cancer patients

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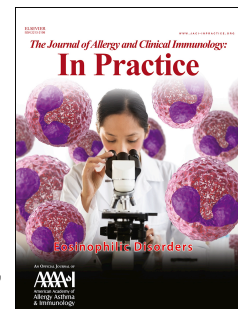
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**Soluble FcεRI, IgE, and tryptase as potential biomarkers of rapid desensitizations for
platin IgE sensitized cancer patients**

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Clinical implications statement (40 words)

Baseline serum sFcεRI titers are potential biomarkers to predict desensitization outcomes.

Desensitization of humanized MCs inhibits IgE-mediated activation correlating with the protection seen during desensitization protocols.

1

2 To the Editor:

3 Platins are effective chemotherapy drugs used to treat ovarian and colon cancers among
4 others, but up to 25% of patients can develop life-threatening IgE-mediated hypersensitivity
5 reactions (HSRs) after multiple exposures, which precludes first line treatment prematurely
6 and impact negatively in patients' quality of life and life expectancy (1-4). Desensitization
7 (DS) protocols induce a temporary tolerance-state in which allergic cancer patients can
8 safely re-introduce their best treatment option. DS are safe procedures but 10-30% of
9 patients experience breakthroughs reactions (1, 5) and the severity of these reactions is
10 unpredictable due to the absence of biomarkers which can identify patients at risk (1, 6).

Mast cells (MCs) are believed to be the main effector cells involved in allergic HSR and DS. Inhibition of acute and late phase mediators, lack of FcεRI receptor internalization and actin rearrangement has been shown in IgE desensitized MCs (7). Recent studies have shown that the soluble FcεRI, sFcεRI, is released during MC activation and prevents anaphylaxis *in vivo* (8). We hypothesized that DS might modulate sFcεRI levels protecting patients against anaphylaxis.

We recruited 14 platin allergic cancer patients undergoing DS with positive skin test (Online Repository text, Table E1). sFcεRI was detectable in all patients and bound to IgE (9). No demographic, serological, or clinical characteristics correlated or modulated sFcεRI titers (Figure E1). To rule out the effect of cancer or chemotherapy, an atopic cohort (Table E2) was compared to cancer patients with no significant differences in sFcεRI or IgE levels (Figure E1). DS successfully allowed all patients to receive their drug target dose. Serum samples obtained before and after completion of DS were analyzed (Figure 1). Tryptase levels were increased in four patients after DS, two of them presenting with breakthrough reactions (patient 1 and 6). IgE levels were increased in eleven patients and sFcεRI increased in nine patients after DS (Table E1).

Based on a recent study (9), 2 ng/mL was used as a cut-off for clinically relevant sFcεRI levels and thus we divided the cohort into two subgroups. In the first subgroup (sFcεRI >2 ng/mL), 80% of the patients (n= 4/5) presented a trend towards increased sFcεRI levels (mean= $32 \pm 0.05\%$), increased IgE, and a significant decrease in tryptase levels after DS (Figure 1B). In the second subgroup (sFcεRI <2 ng/mL), patients presented a trend towards

increased IgE (n= 6/9) and tryptase (n= 4/9) levels, and decreased sFcεRI levels after DS (Figure 1C). Increased tryptase levels were observed in the two patients with breakthrough reactions who presented the highest tryptase titers and the lowest baseline sFcεRI titers (Table E1). When sFcεRI, IgE, and tryptase titers after DS were compared between subgroups, a significant difference in sFcεRI and tryptase levels (Figure 1D-E and Figure E2) was observed in the protected group.

To test our hypothesis of high sFcεRI as a predictor for protection during DS, a blinded clinical follow-up was performed during further DS (Table E3). Overall, breakthrough reactions occurred in 29% of the patients (4/14), 3/4 patients with IgE-mediated type I reactions (3) and 1/4 (patient 14) with a cytokine release reaction (Table E3). The three patients belonged to the at risk subgroup with the lowest sFcεRI titers (Figure E2C).

We investigated the modulation of sFcεRI levels in an *in vitro* DS model of murine humanized MCs. Activation and DS were evaluated using β-hexosaminidase release. β-hexosaminidase and sFcεRI release correlated during activation (Figure E3A-D), and sFcεRI was significantly inhibited during DS by 86% (Figure E3 and Figure 2A-C). Control FcεRIα^{-/-} (MuKO) cells had no β-hexosaminidase and sFcεRI release. Inhibition of sFcεRI release was achieved by cumulative doses of antigen during DS, whereas suboptimal single doses were able to trigger incremental sFcεRI release (Figure E3 and Figure 2D-E). *In vitro* DS successfully rendered humanized MCs unresponsive to activation leading to a lack of sFcεRI release, supporting previous results of the signaling requirements for sFcεRI release (8). Moreover, it might reflect a potential mechanistic

approach to correlate the already known actions of *in vitro* DS and the signaling requirements for sFcεRI release.

To reconcile the *in vitro* results with the *in vivo* data it is likely that complexes of sFcεRI/IgE are protective *in vivo*, decreasing the IgE binding to cell surface FcεRI, preventing MC activation and anaphylaxis. Because sFcεRI is not released without MC activation and during DS, a baseline level of subclinical sFcεRI release is necessary for protection.

We conclude that IgE, sFcεRI, and tryptase levels are useful biomarkers of DS (Figure E4). Patients with sFcεRI levels above 2 ng/mL showed an increase in sFcεRI after DS and a significant decrease in tryptase levels. A protective function of sFcεRI was previously shown in murine models of anaphylaxis, in which administration of recombinant sFcεRI protected against anaphylaxis in naïve mice and diminished response severity in sensitized mice. Patients with higher sFcεRI levels were protected against HSRs during DS and patients with low sFcεRI titers had increased tryptase levels and breakthrough reactions during DS.

Patients who suffered breakthrough IgE-mediated reactions (3/15) were classified as patients at risk and presented sFcεRI levels <2ng/mL. These patients might be at disadvantage as compared with patients with higher sFcεRI levels since free IgE is not captured by sFcεRI and can bind and activate mast cells. These findings point to a delicate balance during DS between IgE and sFcεRI which likely determines the patient's reactivity. Further studies would be needed in a larger population of platin IgE sensitized patients to confirm our findings.

In conclusion, patients with type I platin hypersensitivity phenotype presenting with an endotype with sFcεRI in serum of <2ng/mL can be considered at risk for breakthrough reactions during DS. The use of tryptase, IgE, and sFcεRI serum titers has a promising value in providing personalized care in cancer allergic patients, improving risk assessment. The availability of such biomarkers may be relevant in other immunotherapy protocols.

(990 words)

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FIGURE LEGENDS

Figure 1. Baseline sFcεRI levels as biomarker for risk of IgE-mediated reactions during DS. Outline of in vivo DS protocol of 3bags/12steps (A). Levels of total sFcεRI, total IgE, and tryptase before (black squares) and after (blue circles) DS. Patients (n= 5) with sFcεRI levels >2 ng/mL (B) and patients (n= 9) with sFcεRI levels <2 ng/mL (C) are represented. Levels of total sFcεRI (D) and tryptase (E) before and after DS. 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 (D) as box and whisker graphs (minimum to maximum). Paired t-test or Mann-Whitney test were performed; where *p<0.05, ***p<0.001.

135

136 **Figure 2. Rapid desensitization (DS) of mast cells (MCs) inhibits release of sFcεRI and**
137 **it requires cumulative doses.** Outline of in vitro rapid DS protocol (A). 1×10^6 /mL MCs
138 (humanized murine bone-marrow derived MCs) or MuKO (mFcεRIα^{-/-}) cells were loaded
139 overnight with up to 10% allergic human serum in 200 μL (B, C). Percentage of β-
140 hexosaminidase release (B) and total sFcεRI levels (C) were measured after DS with 0.5
141 μg/mL anti-hIgE. Razine Medium was used as control. 1×10^6 /mL MCs were loaded
142 overnight with 0.5 μg/mL anti-NP cIgE (D, E). Total sFcεRI levels were measured after a
143 single dose challenge or cumulative doses (10 pg/mL to 10 ng/mL NP-BSA) for 10 or 110
144 minutes. Single challenge with 10 ng/mL NP-BSA (Act) or 10 ng/mL BSA (Neg) were
145 used as controls. Data represents mean ± SEM of n=3-8 independent experiments. 1way
146 ANOVA test plus Tukey's multiple correction (B-E) was performed; where *p<0.05,
147 **p<0.01, and ***p<0.001 compared to Neg (B, C) or Act (D, E). □ represents statistics
148 between conditions among Single 110 min data set (D). nd: not detected.

SUPPORTING INFORMATION**Soluble FcεRI, IgE, and tryptase as potential biomarkers of rapid desensitizations
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Conflicts of interest

The authors confirm that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that influenced outcome of the experiments or interpretation of the data.

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Author's contributions

SMR, LV, LAA, EF, and MCC designed experiments, performed research and analyzed data. SMR, LV, and MCC wrote the first draft of the manuscript. ML and ZS assisted with patient data collection. All co-authors contributed to writing the manuscript and approved its final version.

Key words (up to 5-10)

Desensitization; drug allergy; FcεRI; IgE receptor; allergy; mast cell

Abbreviations:

ADRs (adverse drug reactions)

- 46 BSA (bovine serum albumin)
- 47 cIgE (chimeric humanized anti-NP immunoglobulin E)
- 48 CRR (cytokine release reaction)
- 49 DS (desensitization)
- 50 FcεRI (Fc epsilon Receptor I, high affinity IgE Fc receptor)
- 51 hIgE (human Immunoglobulin E)
- 52 hFcεRIα (alpha subunit of human Fc epsilon Receptor I, high affinity IgE Fc receptor)
- 53 IgE (Immunoglobulin E)
- 54 IL-3 (Interleukin-3)
- 55 MC (mast cell)
- 56 mFcεRI (murine Fc epsilon Receptor I, high affinity IgE Fc receptor)
- 57 NP-BSA (4-Hydroxy-3-nitrophenylacetyl bovine serum albumin)
- 58 SCF (stem cell factor)
- 59 sFcεRI (soluble isoform of FcεRI)
- 60 ST (skin test)
- 61

62 **MATERIALS & METHODS**

63 **Reagents**

64 For *in vitro* experiments on murine humanized bone-marrow derived mast cells (MCs),
65 chimeric humanized anti-NP IgE (cIgE) (MCA333S clone JW8/1 Serotec BioRad,
66 Hercules, CA, USA) and its hapten-specific antigen NP-HSA/BSA (N-50051 and N-
67 5050L, Biosearch Technologies, Petaluma, CA, USA) at different concentrations (0.5
68 µg and 0.01-100 ng respectively). Human serum (up to 10%) from an allergic donor

containing IgE against methotrexate (in 200 μ L) followed by α -hIgE challenge (Goat anti-Human IgE-Affinity Purified, Immunology Consultants Laboratory, Inc., Portland, Oregon, USA), was assessed to find the antigen target dose (0.5 μ g, 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 which produces the higher β -hexosaminidase release during challenge). BSA (10 ng) or Razin Medium were used as controls.

Cells and culture conditions

For the generation of MCs, femurs of hFc ϵ RI α^+ /mFc ϵ RI $\alpha^{-/-}$ and mFc ϵ RI $\alpha^{-/-}$ (MuKO) mice were flushed and progenitor cells were cultured as described (1) in RPMI-medium supplemented with 10% FBS (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 10000 U/mL Pen-Strep (Gibco), 2 mM L-Glutamine, 50 μ M β -mercaptoethanol (Gibco), 10 ng/mL recombinant murine IL-3 (Interleukin-3) and SCF (stem cell factor) (PeproTech, Rocky Hill, USA).

Fc ϵ RI cross-linking, sFc ϵ RI 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 μ g/mL) or with medium (in 200 μ L) containing up to 10% serum from patients that were allergic to methotrexate. MCs were stimulated either with single or sequential doses of NP-BSA (0.01-100 ng/mL) or anti-hIgE (0.5 μ g) for 10 or 110 minutes. DS was performed as previously described (2, 3) and outlined in Figure 4.

Culture supernatants were collected and analyzed by ELISA for sFc ϵ RI and β -hexosaminidase levels as previously described (2, 4, 5).

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 (2, 3). 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 4A and Figure 5A.

Mice

Mice were all housed in specific pathogen-free conditions according to the National Institutes of Health (NIH) 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

Cancer patients 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) (6-9) with the culprit drug and any hypersensitivity IgE mediated/mast cell-activation related symptoms (6, 8-14) during platin infusion (1st reaction) from cutaneous to even anaphylaxis. The initial reaction was graded following Brown's classification in: mild/grade - 1 (one symptomatic organ system), moderate/grade - 2 (two or more systems involved), and severe/grade - 3 (more than two systems affected and/or vital sign changes). Presence of other adverse drug reactions (ADR) were reported by the patients.

Serum used in hBMMCs sensitization was collected from a 70 years old male 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 was no history of cancer and allergen-specific IgE (sIgE) levels >0.35 kU_A/L to one or more allergen.

A total of 14 cancer patients 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 desensitization (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 1300-2000g 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 cancer patients

Cancer allergic patients to oxaliplatin or carboplatin entered a drug DS protocol as previously described and as shown in Figure E3. DS protocols with 16 to 12 steps,

escalating the dose 2 to 2.5 times every 15 minutes were applied (6, 8-14). 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 (6). All patients received standard premedication. Omalizumab (anti-IgE therapy) was used in patient number 1, who was highly sensitized and experienced severe reactions during previous DS (Table 1). 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 $\pm$ SEM of the indicated number of individual data points or independent experiments. Correlations were calculated by Pearson rank correlation test and correlation coefficients are displayed as “r”. Statistical analysis was performed using unpaired or paired t-test for comparison between two groups, 1way ANOVA test plus Tukey’s multiple correction for more than two unmatched groups, and 2way ANOVA test plus Tukey’s multiple correction for multiple comparisons between three or more groups. A p value ≤ 0.05 was considered significant.

Word count (1090)

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FIGURE LEGENDS

Figure E1. Levels of sFcεRI in controls and drug allergic cancer patients.

Comparison of sFcεRI or IgE levels in control and cancer patients (A, B). In cancer patients, 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) before (black squares) and after (blue circles) DS. Unpaired t-test or 1way ANOVA test plus Tukey's multiple correction, or Pearson r correlation analysis was performed; where **** $p < 0.0001$. ns: not significant. DS: desensitization.

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). Mann-Whitney test was performed; where $*p < 0.05$.

Figure E3. Activation of MCs triggers release of sFcεRI which is inhibited by cumulative doses. 1×10^6 /mL MCs were loaded overnight with 0.5 μ g/mL anti-NP cIgE (A, B) or up to 10% allergic human serum in 200 μ L (C, D) followed by a single challenge with 100-0.1 ng/mL NP-BSA (A, B) or 2-0.01 μ g/mL anti-hIgE (C, D). 1×10^6 /mL MCs or MuKO (mFcεRI α -/-) cells were loaded overnight with 0.5 μ g/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). 1×10^6 /mL MCs were loaded overnight with 0.5 μ g/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. Single challenge with 10 ng/mL NP-BSA (Act), 10 ng/mL BSA (Neg), or Razin

Medium were used as controls. Data represents mean $\pm$ SEM of n=3-8 independent experiments. 1way ANOVA test plus Tukey's multiple correction was performed; where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$ compared to controls. nd: not detected. * represents statistics between conditions among Single 10 min (H) and cumulative (I) data sets. $\square$ represents statistics between conditions among Single 110 min date set (H).

Figure E4

Soluble Fc ϵ RI 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 Fc ϵ RI release were inhibited upon cumulative doses of antigen

Table E1. Clinical and serological characteristics of cancer patients. §Patients 1 and 6 experienced a mild (grade 1) reaction during DS. Patient 1 had received Omalizumab as DS premedication. Values of total sFc ϵ RI, 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 sFc ϵ RI above 2 ng/mL are marked with *. F: female; M: male; AA: allergic asthma; AR: allergic rhinitis; AC: allergic conjunctivitis; CD: contact dermatitis; FA: food allergy; ADR: other adverse drug reaction; C: carboplatin; O: oxaliplatin; ST: skin test; DS: desensitization; SD: standard deviation.

Table E2. Clinical and serological characteristics of control group. Atopy was described as allergen-specific IgE sensitization to one or more allergen without clear allergic symptoms. F: female; M: male; HDM: house dust mite; SD: standard deviation.

Table E3. Clinical characteristics of follow up on cancer cohort. Patients with sFcεRI titers above 2 ng/mL are marked with *. CRR: cytokine release reaction; DS: desensitization; IL-6: interleukin 6; Y: yes; N:no; ?: unknown.

Table E1

Patient	Age (years)	Gender	IgE disease	ADR	Cancer type	Drug	Infusion at which reaction occurred	Grade of 1st reaction	ST	N° DS	Reaction during DS	sFceRI (ng/mL)			IgE (kU/L)			Tryptase (ng/mL)		
												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%

Table E2

Patient	Age (years)	Gender	Disease	Relevant allergen	sFceRI (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

Patient	DS at consent	Total N° DS	Reaction during further DS	N° Reactions	DS N°	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		

Table E3



Soluble FcεRI , IgE, and tryptase as biomarkers of rapid desensitizations for platin IgE sensitized cancer patients

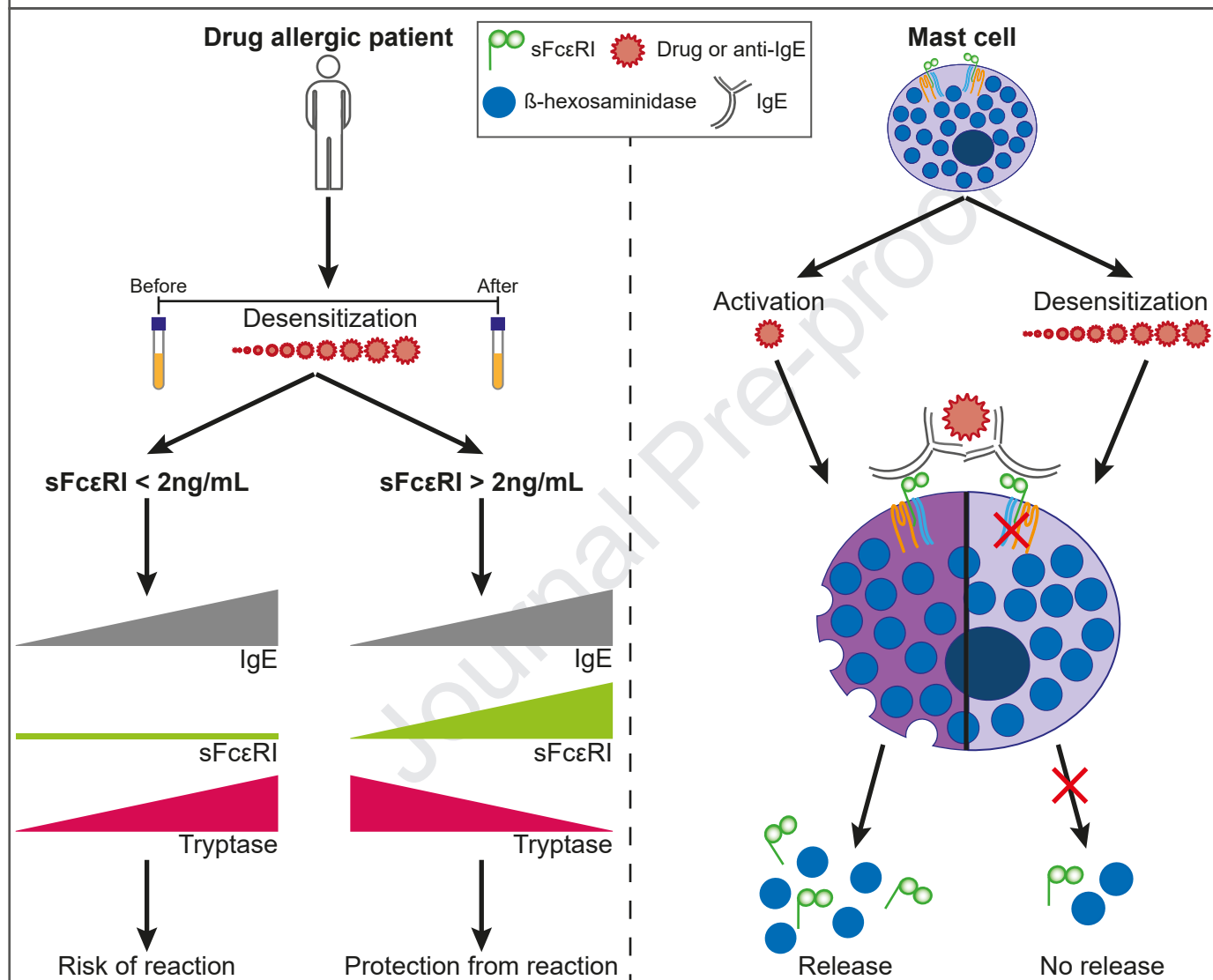


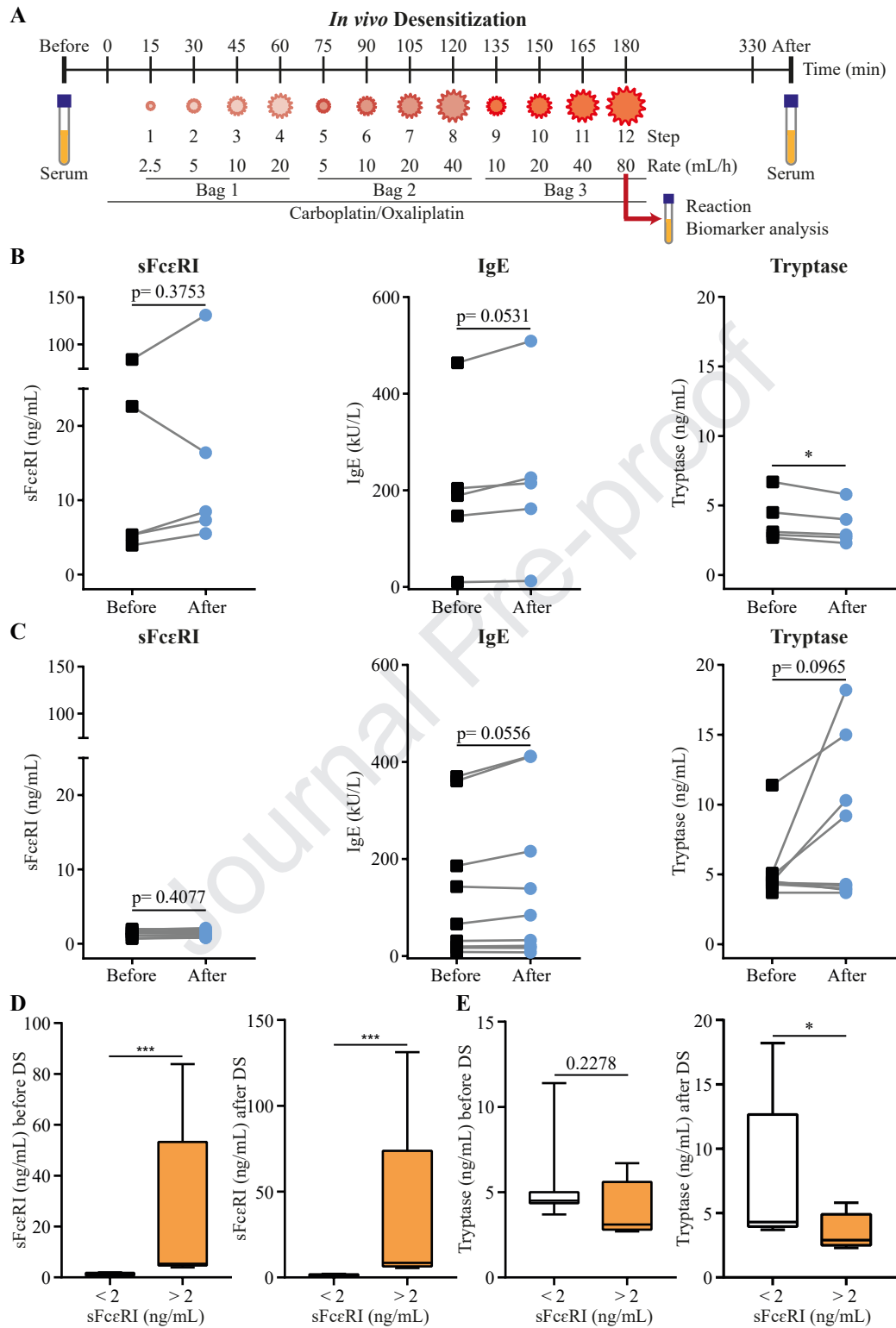
Figure 1

Figure 2

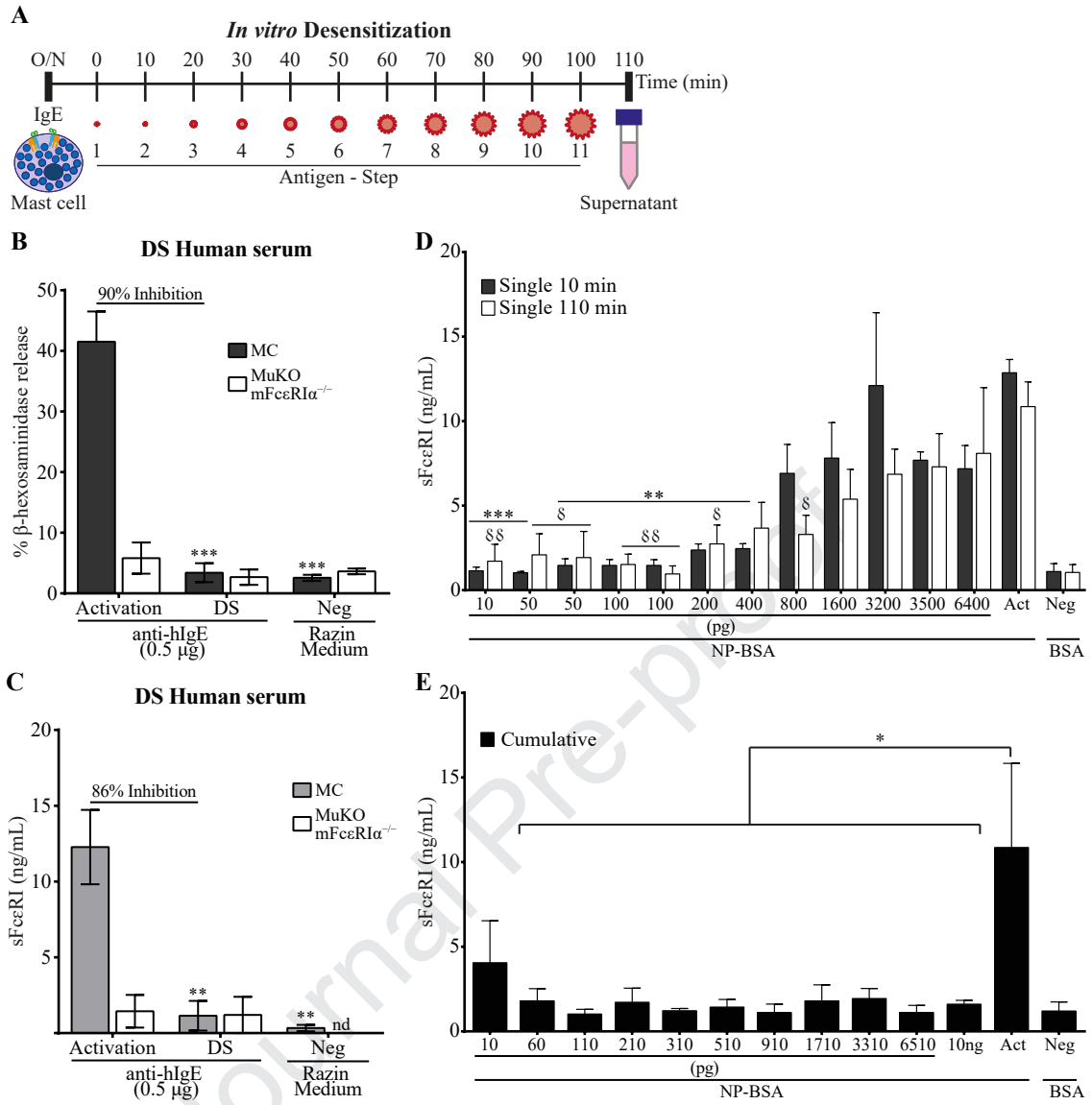


Figure E1

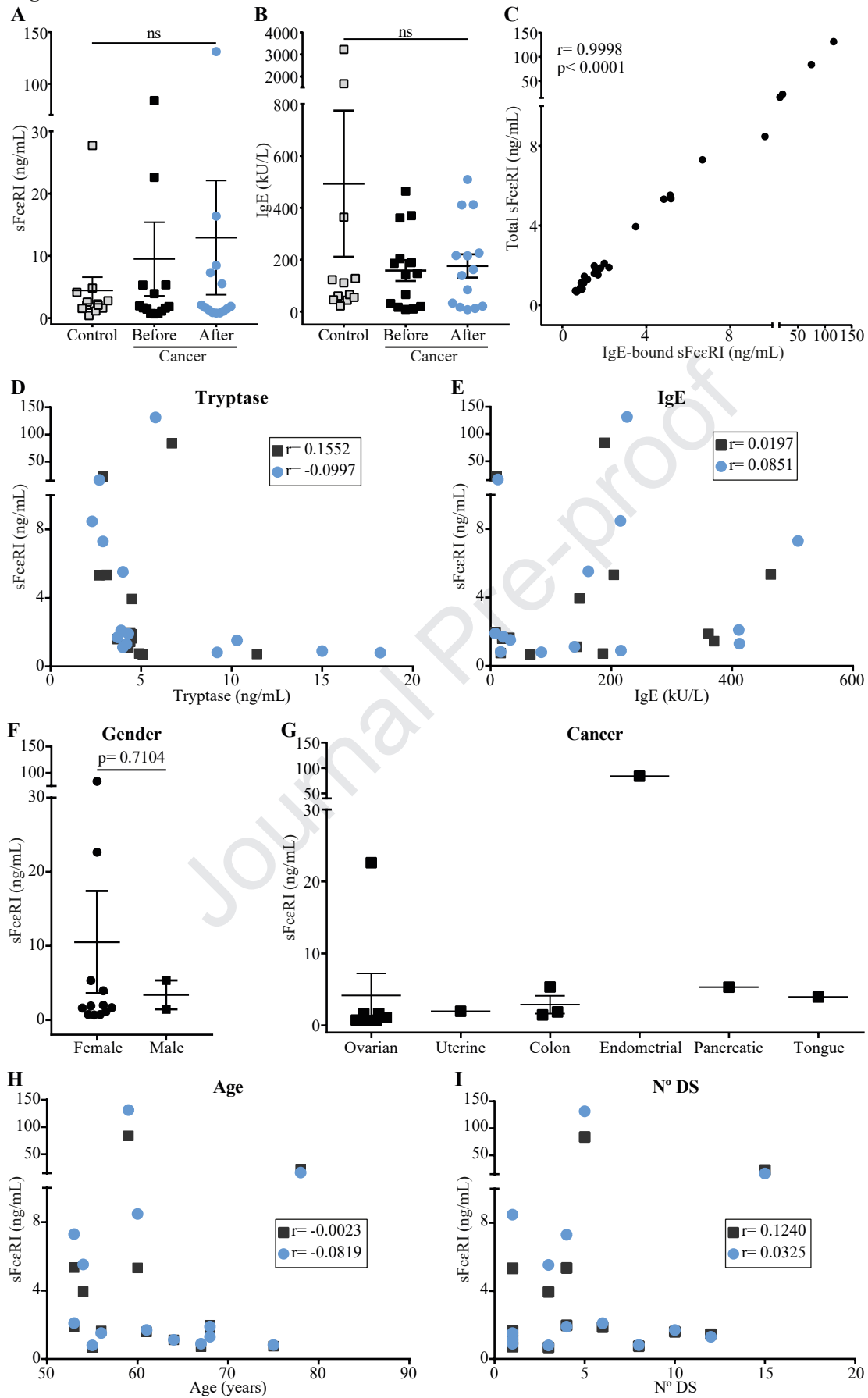


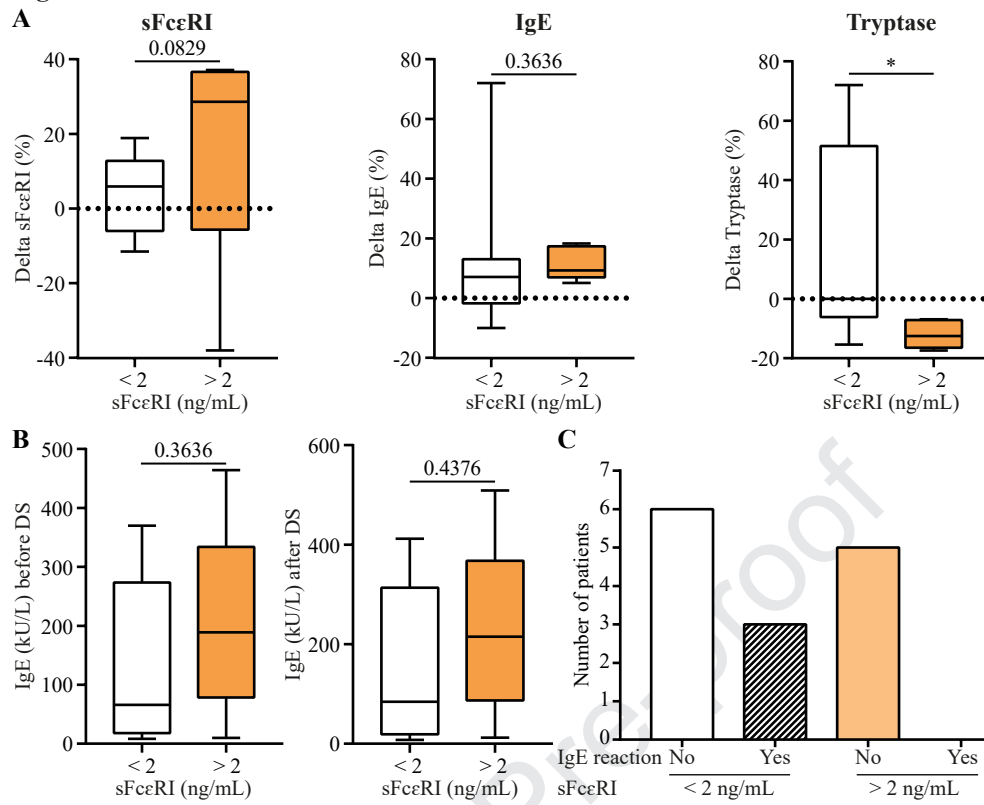
Figure E2

Figure E3

