



Research Paper

Extracorporeal IgE Immunoabsorption in Allergic Asthma: Safety and Efficacy



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ABSTRACT

Background: Prevention of IgE-binding to cellular IgE-receptors by anti-IgE (Omalizumab) is clinically effective in allergic asthma, but limited by IgE threshold-levels. To overcome this limitation, we developed a single-use IgE immunoabsorber column (IgEnio). IgEnio is based on a recombinant, IgE-specific antibody fragment and can be used for the specific extracorporeal desorption of IgE.

Objective: To study safety and efficacy of IgEnio regarding the selective depletion of IgE in a randomized, open-label, controlled pilot trial in patients with allergic asthma and to investigate if IgEnio can bind IgE-Omalizumab immune complexes.

Methods: Fifteen subjects were enrolled and randomly assigned to the treatment group (n = 10) or to the control group (n = 5). Immunoabsorption was done by veno-venous approach, processing the twofold calculated plasma volume during each treatment. A minimum average IgE-depletion of 50% after the last cycle in the intention-to-treat population was defined as primary endpoint. Safety of the treatment was studied as secondary endpoint. In addition, possible changes in allergen-specific sensitivity were investigated, as well as clinical effects by peak flow measurement and symptom-recording. The depletion of IgE-Omalizumab immune complexes was studied *in vitro*. The study was registered at clinicaltrials.gov (NCT02096237) and conducted from December 2013 to July 2014.

Results: IgE immunoabsorption with IgEnio selectively depleted 86.2% ($\pm 5.1\%$ SD) of IgE until the end of the last cycle ($p < 0.0001$). Removal of pollen allergen-specific IgE was associated with a reduction of allergen-specific basophil-sensitivity and prevented increases of allergen-specific skin-sensitivity and clinical symptoms during pollen seasons. IgEnio also depleted IgE-Omalizumab immune complexes *in vitro*.

The therapy under investigation was safe and well-tolerated. During a total of 81 aphereses, 2 severe adverse events (SAE) were recorded, one of which, an episode of acute dyspnea, possibly was related to the treatment and resolved after administration of antihistamines and corticosteroids.

Conclusions: This pilot study indicates that IgE immunoabsorption with IgEnio may be used to treat patients with pollen-induced allergic asthma. Furthermore, the treatment could render allergic patients with highly elevated IgE-levels eligible for the administration of Omalizumab and facilitate the desorption of IgE-Omalizumab complexes. This study was funded by Fresenius Medical Care Deutschland GmbH, Bad Homburg, Germany.

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Abbreviations: AUC, area under the curve; AV, visit of apheresis group; BAT, basophil activation-testing; CV, visit of control group; IC, informed consent; IgE, immunoglobulin E; pt./pts., patient/s; SD, standard deviation; VAS, visual analogue scale.

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1. Introduction

IgE (immunoglobulin E)-associated allergies are a major health threat in industrialized countries affecting more than 25% of the population (Pawankar et al., 2012). Asthma represents one of the most severe manifestations of allergy because of its chronic course, strong impact on quality of life and severe, life-threatening symptoms (Holgate, 2012). Sensitization to airborne allergens from indoor allergen sources such as house dust mite, pets, mold and cockroach is a causative factor for asthma (Reed et al., 1985; Pollart et al., 1989; Konradsen et al., 2015). Furthermore, severe exacerbations are frequently observed after seasonal exposure to allergens from birch, grass, cypress and ragweed pollen (Pollart et al., 1988; Suphioglu et al., 1992; Schäppi et al., 1997; Midoro-Horiuti et al., 1992).

In allergic asthma, symptoms are caused by allergen-induced cross-linking of IgE antibodies on the surface of mast cells and basophils and the subsequent release of inflammatory mediators (Holgate, 2012). IgE may also play a major role in T cell activation through IgE-facilitated allergen presentation (Mudde et al., 1990; van der Heijden et al., 1993). T cell-derived cytokines such as IL-5 (Interleukin 5) and IL-13 contribute to mucus secretion and eosinophilic infiltration, respectively, both of which are involved in airway remodeling (Holgate, 2012). Since IgE antibodies play a central role in allergic asthma, several therapeutic strategies targeting IgE or the underlying Th2 (T helper 2 cell) immune response have evolved or are currently in clinical evaluation (Holgate, 2014). These strategies target cytokines which are involved in Th2-driven allergic asthma (Wenzel et al., 2013; Bel et al., 2014; Ortega et al., 2014; Gauvreau et al., 2014) or focus directly on IgE antibodies (O'Byrne and Tworek, 2015; Molimard et al., 2010; Humbert et al., 2014). Omalizumab, a therapeutic anti-IgE antibody preventing IgE from binding to its high-affinity receptor (FcεRI), is effective for the treatment of severe allergic asthma. According to clinical studies, it reduces exacerbations and the need for steroids (Molimard et al., 2010). One major drawback of injected Omalizumab is that its applicability is limited by a weight-dependent maximum level of total IgE up to which it can be administered, i.e., 1500 U/ml within the EU (EMA, 2015) and 700 U/ml in the US (FDA, 2015), respectively. This precludes many polysensitized patients with severe symptoms from anti-IgE treatment. In the present study, we report results from a clinical trial where an IgE-immunoadsorber, termed IgEnio, was used to treat subjects suffering from allergic asthma. Efficacy of IgE-depletion and safety of the treatment were studied as primary and secondary endpoints, respectively. In addition, several immunological and clinical parameters were assessed to investigate possible effects of the treatment on IgE-driven mechanisms.

2. Materials and Methods

2.1. Description of the Medical Device IgEnio

IgEnio (Fresenius Medical Care, Bad Homburg, Germany) is a single-use, IgE-specific immunoadsorber column based on a single-chain variable fragment, ScFv12 (Lupinek et al., 2009) (BM10, Biomay, Vienna, Austria), for the selective removal of IgE from plasma of allergic patients by extracorporeal immunoadsorption. ScFv12 is specific for IgE, does not cross-link IgE on mast cells and basophils (Lupinek et al., 2009) and is produced in *E. coli* under GMP (good manufacturing practice) conditions.

2.2. Study Design

The study was designed in accordance with the declaration of Helsinki as a prospective, randomized, controlled, open-label trial and was performed in one study-center at the Medical University of Vienna, Austria. The trial was approved by Austrian health authorities (AGES),

by the ethics committee of the Medical University of Vienna and was registered at clinicaltrials.gov (NCT02096237) (FMC, 2014).

Men and women, aged 18–50 years, who were suffering from allergy-driven asthma and with a minimum total IgE-level of 300 U/ml were eligible for participation. Diagnosis of allergic asthma was established by patient's history, measurement of allergen-specific IgE, skin prick testing (SPT) and by bronchial provocation with methacholine. Asthma-severity was graded according to guidelines of the German Medical Association (Bundesärztekammer, 2009) that are based on GINA-guidelines (GINA, 2007), the British Thoracic Society guidelines on the management of asthma (BTS, 2010) and guidelines for the diagnosis and management of asthma of the National Heart, Lung, and Blood Institute (NHLBI, 2007). Subjects suffering from non-allergic asthma, receiving Omalizumab-therapy or having received allergen-specific immunotherapy in the last 6 months were excluded from participation (FMC, 2014). Fifty years were selected as upper age level to reduce co-morbidities which would have precluded immunoadsorption.

After enrollment of 15 eligible subjects, participants were ranked according to total-IgE levels and, in groups of three, randomly assigned to the apheresis ($n = 10$) or to the control group ($n = 5$; Fig. 1a) at a 2:1 ratio. This procedure facilitated achievement of comparable median total IgE-levels in the two groups.

Treatment cycles consisted of 3 immunoadsorptions within one week and were followed by intervals of 4 weeks without intervention. Each patient was subjected to 3 cycles, i.e., 9 aphereses in total (Fig. 1b). Control visits were scheduled two weeks after the last treatment of each cycle, the final visit was planned for the 5th week after the last cycle. Subjects allocated to the control group were seen accordingly, but with 2 visits only in those weeks that corresponded to treatment weeks. Total study-duration was approximately 16 weeks for each patient.

The primary endpoint of the trial was defined as $\geq 50\%$ reduction of total IgE after the last, i.e., the 9th immunoadsorption with IgEnio under the treatment scheme described above, when related to the pre-value of the first treatment. As secondary endpoint, safety of the treatment was investigated by recording adverse events and serious adverse events in accordance with ISO 14155 and MedDRA (<http://www.meddra.org>), and by analysis of blood and urine samples (Interlab GmbH Central Lab Services, Munich, Germany) (GCP, 2011). In order to detect possible effects of IgE-desorption on IgE-mediated mechanisms, additional immunological and clinical parameters were recorded (Table 1).

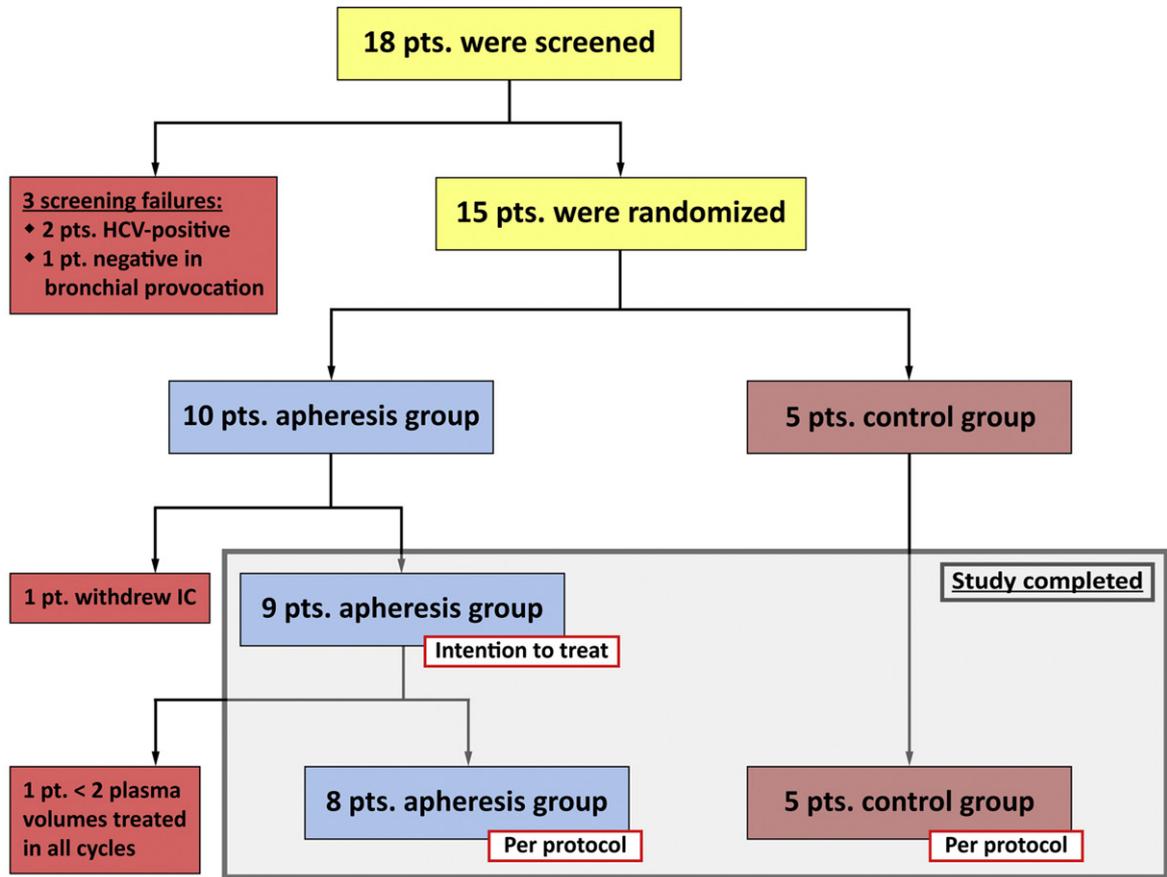
At every treatment visit, blood was collected immediately before and after apheresis. From control patients, one blood sample was obtained at corresponding visits (Fig. 1b). At screening, control- and follow-up visits, all patients were subjected to clinical examinations including lung function assessment, titrated skin prick testing, urine- and blood-collection. For the complete study duration, patients recorded results from daily peak flow measurements (Vitalograph peak-flow-meter, North Buckinghamshire, UK), symptom severity (general well-being graded by visual analogue scale [VAS]; eye-, nose- and lung-specific symptoms: severe, moderate, mild or no symptoms) and eventual adjustments of previously prescribed medication in patient diaries. At the beginning and at the end of the trial, patients completed a validated asthma control questionnaire (Juniper et al., 1999).

The complete study protocol is provided in the online repository.

2.3. Apheresis Treatment

Every week, 2 subjects were treated simultaneously by immunoadsorption. Blood was drawn via an 18-gauge needle from a peripheral vein at a flow rate of 50–80 ml/min. Anticoagulation during treatment was maintained by continuous citrate dosage (ACD-A, anticoagulant citrate dextrose, formula A; Fresenius Kabi, Bad Homburg, Germany) at a volume/citrate ratio of 1:25, and sodium heparin (1000–1500 IU/h, Heparin Immuno, Immuno AG, Vienna, Austria). Plasma was separated

a



b

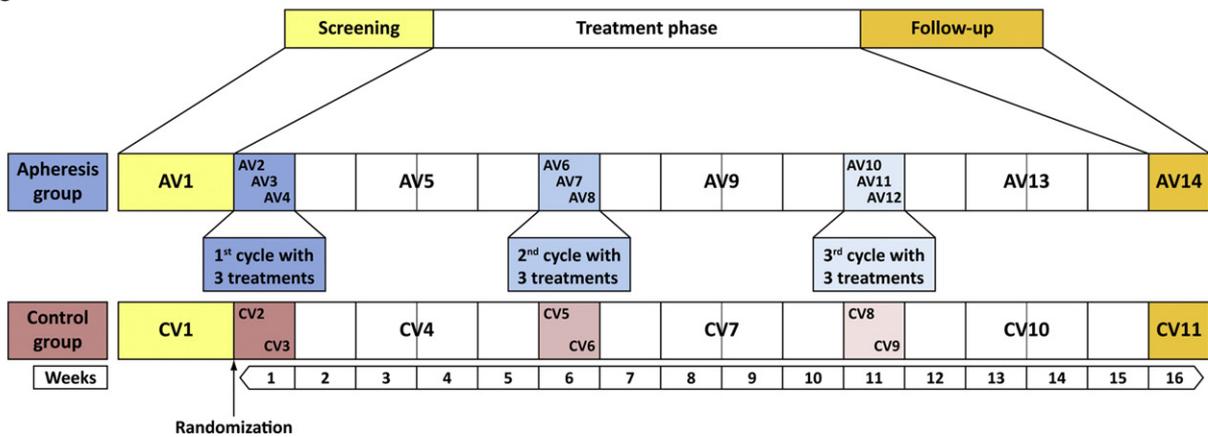


Fig. 1. Enrollment, randomization and study design. (a) After screening (yellow; AV1, CV1 in b), subjects were allocated to the apheresis (blue) or the control group (pink). (b) Treatment-visits (AV2, AV3, etc.) are indicated by blue boxes, corresponding visits of the control group (CV2, CV3, etc.) by pink boxes. Intervals between treatment weeks, including control visits (AV5, CV4, etc.) are shown in white. The study was completed after a follow-up visit in week 16 (orange; AV14 and CV11). Weeks after randomization are shown at the bottom. pt./pts. – patient/s; IC – informed consent; AV – visit apheresis group; CV – visit control group.

from blood cells by centrifugation (COM.TEC, Fresenius Kabi, Bad Homburg, Germany). Mean blood and plasma flow rates were 55.2 ml/min (± 6.1 ml/min SD) and 29.9 ml/min (± 0.5 ml/min SD), respectively, requiring an average time of 3.5 h (± 0.9 h SD) to treat the twofold plasma volume of the respective patient as calculated using the formula according to Sprenger et al. (1987). Mean deviation from plasma volume planned to be treated was -10.7% ($\pm 10.1\%$ SD) for all 81 treatments.

Separated plasma was conducted by a COM.TEC plasma pump from the centrifugal separation chamber directly to the IgE-adsorber where adsorption of IgE occurred. The processed plasma was thereafter passed through a particle filter as second safety barrier against accidental particle infusion, and reunified with the blood cells in the bubble catcher of the cell separator's return line. From there, it was returned to the patient by a second peripheral venous access at the opposite arm as used for

Table 1
Clinical and immunological parameters.

Primary variable: reduction of total IgE by $\geq 50\%$
Total IgE (ImmunoCAP)
Secondary variables: safety parameters
Adverse events (AE), serious adverse events (SAE)
Coagulation parameters: aPTT, Fibrinogen, Factor VIII, ionized calcium, Thrombin-Antithrombin III complex (TAT)
Complement-activation: C3a, C5a
Electrolytes (serum): Na, K, total Ca, PO ₄ , Cl
Blood gas analysis: acid-base status, O ₂ -saturation
Hematology: white blood cells, erythrocytes (number, MCV, MCH, MCHC), hemoglobin, hematocrit, platelets
Liver and kidney parameters: AST, ALT, γ -GT, creatinine, bilirubin, uric acid, BUN
Urine analysis: pH, leukocytes, erythrocytes, bilirubin, urobilinogen, ketone bodies, nitrite, glucose
Blood lipids: LDL, HDL, triglycerides
Bone metabolism: alkaline phosphatase
Hemolysis: lactate dehydrogenase
Protein loss: albumin
Inflammation/Infection: CRP (C-reactive protein)
Heart and muscle injury: CK (creatin kinase)
Additional clinical parameters:
Lung function parameters: PEF, FEV ₁
Asthma control questionnaire
Patient's diary: symptoms, medication
Skin prick test
Body temperature
ECG
During treatment:
Treatment time
Blood flow/plasma flow
Processed plasma volume
Heart rate
Blood pressure (every 60 min)
Additional immunological parameters:
IgE and IgG reactivity profiles (ISAC, Thermo Fisher Scientific)
Specific IgE-levels (ImmunoCAP, Thermo Fisher Scientific)
Allergen-induced basophil activation (CD203c upregulation)
CD23-expression on B cells in peripheral blood
IgE positive cells among PBMCs (peripheral blood mononuclear cells)
Fc ϵ RI-expression on PBMCs
Allergen-induced T cell activation and cytokine secretion

blood collection. At the end of treatment residual blood in the blood lines and the adsorber was displaced by 0.9% saline and re-infused to the patient.

Since this clinical trial was conducted as a pre-CE study, apheresis treatments were performed using clinical samples of IgEnio.

2.4. Measurement of Total and Allergen-specific IgE

All serum samples were tested by ImmunoCAP (Thermo Fisher/Phadia AB, Uppsala, Sweden) for levels of total IgE and for IgE specific to defined seasonal (birch, ash, timothy grass, mugwort, *Alternaria*) and perennial allergen sources (*Aspergillus*, house dust mite, cat, dog). Results are reported in U/ml for total IgE, and in UA/ml for allergen-specific IgE (Bazal and Hamburger, 1972). Relative IgE-depletion rates were calculated for each treatment day and cycle, respectively, as described below.

2.5. Measurement and Calculation of Changes in Concentrations of Serum IgG, IgA, IgM and Albumin

Levels of total IgG, IgA and IgM were measured in samples obtained before and immediately after apheresis treatments on a BNI nephelometer using N Antisera to human IgG, IgA and IgM (Siemens Healthcare Diagnostics, Vienna, Austria). Relative changes of antibody-concentrations were calculated for each treatment by relating post- to the respective pre-treatment values. In addition, mean changes were calculated for every apheresis cycle. Relative changes in serum albumin (Interlab GmbH Central Lab Services) were calculated accordingly and compared

to results obtained for IgG, IgA and IgM in order to assess if decreases of antibody levels were due to interaction with the immunoabsorber or to serum dilution caused by infusion of electrolyte solution during apheresis.

2.6. Titrated Skin Prick Testing

At screening, control and follow-up visits, all patients were subjected to titrated skin prick testing (SPT) for one or two allergen sources, depending on individual sensitization profiles. Patients were asked to pause intake of antihistamines at least 48 h before skin prick testing. In case this was not possible due to severity of symptoms, SPT was omitted (Heinzerling et al., 2013).

Extracts from dog dander, house dust mite, birch pollen and *Alternaria alternata* were purchased from Allergopharma (Reinbek, Germany), cat dander and grass pollen extracts from Stallergenes (Antony, France). Dilutions of extracts were freshly prepared on the day of skin prick testing in sterile physiological NaCl-solution (Fresenius Kabi Austria GmbH, Graz, Austria). Extracts were diluted from 1:2 to 1:128 at twofold steps and pricked in duplicates on the patients' forearms, in parallel to undiluted extracts, NaCl solution and histamine-control. After 20 min, wheals were marked by felt pen and transferred to SPT-documentation forms using adhesive tape. Wheal areas were quantified by planimetry using ImageJ software, version 1.48 (Rasband, 1997–2014). The sum of mean wheal areas from duplicates was calculated for all wheals induced by dilutions of the same extract in the respective patient. Relative changes of total wheal areas were calculated using results from the screening visit as baseline.

2.7. Basophil Activation Experiments

Lyophilized extracts from grass pollen mix, cat and dog dander were purchased from Stallergenes (Antony, France), lyophilized birch pollen extract from Allergopharma (Reinbek, Germany). Extracts were reconstituted in sterile physiological NaCl solution (Fresenius Kabi Austria GmbH). Raw material for the preparation of an extract from *Dermatophagoides pteronyssinus* was obtained from Allergon (Allergon AB, Ångelholm, Sweden) and an aqueous extract in phosphate buffered saline (PBS) was prepared (Banerjee et al., 2015). Aliquots of all extracts were stored in glass vials at -20°C . For each basophil activation test, series of tenfold dilutions in physiological NaCl or PBS, respectively, ranging from 1 $\mu\text{g/ml}$ to 10^{-6} $\mu\text{g/ml}$ were freshly prepared.

Triplicates of 45 μl from heparinized blood samples that were collected during screening, control and final visits were incubated with 5 μl of extract dilutions. Every patient was tested for basophil reactivity with extracts from the same allergen sources that were also used for SPT in this patient. As controls, anti-IgE antibody clone E124.2.8 (Beckman Coulter, Fullerton, CA, USA, RRID:AB_1575992), PBS and isotype-matched antibodies (phycoerythrin (PE) or fluorescein isothiocyanate (FITC)-labeled mouse IgG₁/k isotype control, clone MOPC-21, BD Biosciences, San Jose, CA, USA, RRID: AB_396091 and RRID: AB_396090, respectively) were used (Hauswirth et al., 2002). In addition, blood aliquots were incubated with 2.5 μl of PE-labeled CD203c mAb clone 97A6 (Beckman Coulter, RRID:AB_141295) and 2.5 μl of FITC-labeled CD63 mAb clone CLBGran/12 (Beckman Coulter, Art.-nr. IM1165U) for 15 min at 37°C . Thereafter, samples were subjected to erythrocyte lysis with 1 ml of FACS Lysing Solution (BD Biosciences). Then, cells were washed, resuspended in PBS, and analyzed by means of 2-colour flow cytometry on a FACScan (BD Biosciences). Basophils were detected on the basis of forward side-scatter characteristics and of CD203c-expression and analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

Allergen-induced upregulation of CD203c and CD63 was calculated from mean fluorescence intensities (MFIs) obtained from stimulated (MFI_{stim}) and unstimulated (MFI_{control}) cells and expressed as stimulation index (MFI_{stim}/MFI_{control}). Areas under the curve (AUC) for

stimulation indices of all dilution steps tested were calculated. Using results from the screening visit as baseline, relative changes in basophil reactivities were determined.

2.8. In Vitro Depletion of IgE From Sera After Addition of Omalizumab

Sera from two different allergic donors with moderately and highly elevated total IgE-levels (i.e., serum 1: 263 U/ml; serum 2: 1427 U/ml), respectively, were collected. Omalizumab (Novartis, Basel, Switzerland) was added to aliquots of each sample at an IgE-to-Omalizumab ratio of 1:1 or 1:40, and incubated at room temperature for 1 h on a rocker. As reference, aliquots of both sera without Omalizumab were analyzed.

Five milliliter aliquots of each serum sample were applied to columns containing 2 ml of IgEnio adsorber material, flow-through was collected in 1 ml aliquots. Total IgE-levels were measured by ImmunoCAP (Thermo Fisher) before application to the column and in the last aliquot of each flow-through. For each sample the percentage of IgE-depletion after single passage through the column was calculated.

2.9. Additional Immunological Parameters

In blood samples collected at screening, control and follow-up visits, expression of IgE-receptors (FcεRI and CD23) on basophils and B cells, respectively, and allergen-induced T cell activation were studied. Details are provided in the online repository.

2.10. Statistical Methods

Sample size calculation was based on a one-sided *t*-test with a one-sided significance level of $\alpha = 0.025$. Assuming a mean IgE reduction rate of 70% and a standard deviation of 17%, a sample size of $n = 8$ provided 80% statistical power to prove that the margin of 50% (primary endpoint) was significantly surpassed. To account for an expected drop-out rate of up to 20%, a total of 10 patients were included in the apheresis group, resulting in a final study population of $n = 15$, including 5 control patients.

All data collected in CRFs and patient diaries were entered into a ClinTrials database using double data entry. After database lock, complete data were exported to SAS V9.2 for statistical analysis. Prick test data were transmitted to the sponsor (Fresenius Medical Care, Bad Homburg, Germany) via Microsoft Excel and imported to ClinTrials database, as were transport files from the external laboratory (Interlab GmbH Central Lab Services, Munich, Germany). For generation of random codes SAS was used. Sample size estimation was performed with nQuery Advisor 5.0.

2.10.1. Primary Analysis

Primary analysis was based on the intention-to-treat (ITT) population. Additional analysis of IgE data of per-protocol (PP) patients was performed to assess validity of results (data not shown). Safety analyses were performed on the safety population.

For primary statistical analysis, the null hypothesis (H_0), i.e., IgE reduction after 3 cycles of apheresis (in total, 9 treatments) is $<50\%$, was tested against the alternative hypothesis (H_1), i.e., IgE reduction after 3 cycles of apheresis (in total, 9 treatments) is $\geq 50\%$. Statistical analysis was based on IgE assessments before treatment 1 of the first cycle, AV2pre (IgEpre; AV – apheresis visit), and after treatment 3 of the last cycle, AV12post (IgEpost). The percentage of IgE reduction (RR – reduction rate) was calculated as

$$RR = (\text{IgEpre} - \text{IgEpost}) / \text{IgEpre} \times 100$$

A one-sided, one-sample *t*-test was calculated on all IgE reduction rates in the apheresis group and H_0 was rejected if $p < 0.025$. Since

primary analysis was based on a single statistical test no adjustment for multiplicity was necessary.

2.10.2. Secondary Analysis

For secondary analysis of changes in total IgE-levels, reduction rates were calculated as denoted above: For each treatment (pre- versus post-value of the same treatment) and for each cycle (pre-value of the first treatment to post-value of the third treatment of the same cycle). In case of single missing IgE data (pre- or post-treatment), the complete treatment was excluded from statistical analysis. Confidence intervals were calculated for reduction rates, incorporating the repeated measures design where applicable.

For the control group, relative changes of total IgE-levels were calculated for those weeks that corresponded to treatment weeks of the apheresis group (Fig. 1b). Comparisons between apheresis and control group were calculated employing linear mixed models.

For clinical outcome data, including prick test and lung function test, descriptive summary tables at study start and study end, as well as for differences between start and end of the trial were generated for the apheresis and the control group.

For the Asthma Quality of Life Questionnaire, data analysis was performed according to validated recommendations (Juniper et al., 1999). Statistical analysis was performed on inter-group differences as well as intra-group changes of the overall score. Patient diary data were analyzed accordingly.

Statistical comparisons were performed according to the measurement level of each parameter (*t*-test, Wilcoxon test, Fisher's Exact test). All *p*-values calculated in the context of secondary analyses were two-sided and considered purely descriptive and exploratory.

3. Results

3.1. Study Population

Patients were enrolled from December 2013 to February 2014. Follow-up visits were completed in July 2014. Among 18 patients who had given informed consent at the screening visit, 2 were excluded from participation due to positive serological results to HCV and one because of absence of bronchial hyperresponsiveness in methacholine provocation testing (Fig. 1a). After enrollment of 15 eligible subjects was completed, participants were ranked according to their total IgE-levels. Starting with those patients with the highest total IgE values, randomization to the apheresis ($n = 10$) or to the control group ($n = 5$) was done in groups of 3 subjects each at a 2:1 ratio. After randomization and before the first immunoadsorption treatment was started, one patient of the treatment group withdrew informed consent for personal reasons. All remaining 14 individuals completed the trial (Fig. 1a, Table 2).

Due to this randomization algorithm, both groups were comparable in terms of total IgE-levels. In the apheresis group, median asthma-severity was higher than in the control group, but this difference was statistically not significant ($p = 0.62$; Table 3).

The highest total IgE level at the screening visit (AV1/CV1) was 3582 U/ml (Table 2). No subjects suffering from allergic asthma with higher IgE-levels could be recruited in our study. This is in accordance with results obtained for patients with allergic asthma in different countries where IgE-levels in such populations were usually lower than 10,000 U/ml (Gergen et al., 2009; Davila et al., 2015; Manise et al., 2016; Tay et al., 2016).

3.2. Reproducible and Selective Depletion of 87.3% of total IgE per Cycle of Immunoadsorption with IgEnio

Mean IgE-depletion rates were 78.1% ($\pm 5.4\%$ SD, $p < 0.0001$) per treatment and 87.3% ($\pm 3.3\%$ SD, $p < 0.0001$) per cycle (Fig. 2a, Table 4). Despite a wide range of individual pre-treatment IgE-levels (AV2pre:

Table 2
Characterization of study subjects.

Pat. Id.	Group	Sex	Age	total IgE [U/ml]	Clinical symptoms	Allergic sensitizations	Comments
002	A	F	32	3582	R, C, A (3), AD	Phl, Can, Fel, Equ, Mus, Hev, Asp, Bos, Gad	Recurrent systemic anaphylactic reactions following consumption of milk and dairy products. Severe AD.
003	A	M	45	340	R, C, A (3)	Bet, Phl	Spends approx. 3 months per year abroad to avoid exposure to birch pollen which causes severe respiratory symptoms.
005	A	M	39	304	R, A (3)	Bet, Fra, Phl, Amb, Fel	Receives high-dosage corticosteroid injection annually.
006	A	M	26	602	A (1), AD	Bet, Fra, Phl, Fel, Can, Mus, Der	Severe AD.
009	A	M	20	894	R, C, A (3)	Bet, Fra, Phl, Fel, Equ, Mus, Alt, Der	Recurrent severe asthma attacks. Between screening visit and first apheresis hospitalized for several days in intensive care unit due to intractable status asthmaticus.
011	A	F	24	592	A (1)	Bet, Fra, Phl, Pla, Can, Fel, Equ, Der	
012	A	F	29	347	R, A (4)	Phl, Can, Fel, Equ, Der	
017	A	M	49	646	R, A (3), AD	Bet, Fra, Phl, Art, Fel	Severe AD.
018	A	F	22	323	A (1), AD	Bet, Fra, Phl, Fel, Equ, Der	
004	C	M	37	554	R, C, A (4)	Bet, Fra, Phl, Art, Fel, Alt, Der	Recurrent severe asthma attacks.
010	C	M	22	1987	R, C, A (1), AD	Phl, Can, Fel, Equ, Mus, Alt, Asp	
013	C	M	19	290	R, A (1)	Phl, Pla, Fel, Der	
014	C	F	22	674	A (1)	Bet, Can, Der	
016	C	M	19	596	A (3)	Der	Hospitalized every autumn for several weeks due to deterioration of allergic asthma.

Pat. Id.: patient-identification; Groups: A – apheresis (blue boxes), C – control (pink boxes); Sex: M – male, F – female; Age: age when informed consent was given; Clinical symptoms: R – allergic rhinitis, C – allergic conjunctivitis, A (grade of severity) – allergic asthma, AD – atopic dermatitis; Allergic sensitizations: Bet – birch, Fra – ash tree, Phl – grass, Amb – ragweed, Art – mugwort, Pla – plantain, Can – dog, Fel – cat, Equ – horse, Mus – mouse, Der – house dust mite, Asp – *Aspergillus*, Alt – *Alternaria*, Hev – latex, Bos – cow's milk, Gad – codfish.

Table 3
Characterization of study groups.

	Apheresis-group (n=9)			Control-group (n=5)		
	mean	SD	min - med - max	mean	SD	min - med - max
Sex (male)	n=5 (55.6%)			n=4 (80%)		
Age [years]	32.1	10.3	20-30-49	24.2	7.4	19-23-37
Body weight [kg]	68.1	18.9	47-55-95	67.6	9.4	55-65-80
Body height [cm]	172.3	14.8	156-165-195	175.6	6.2	165-179-180
BMI [kg/m²]	22.48	2.96	19.1-22.6-27.8	21.84	1.98	20.2-21.2-25
Total IgE [U/ml]	847.8	1044.3	304 - 592 - 3582	820.2	668.0	290 - 596 - 1987
Asthma-score	2.4	1.1	1 - 3 - 4	2	1.4	1 - 1 - 4

SD – standard deviation; min – minimum; med – median; max – maximum.

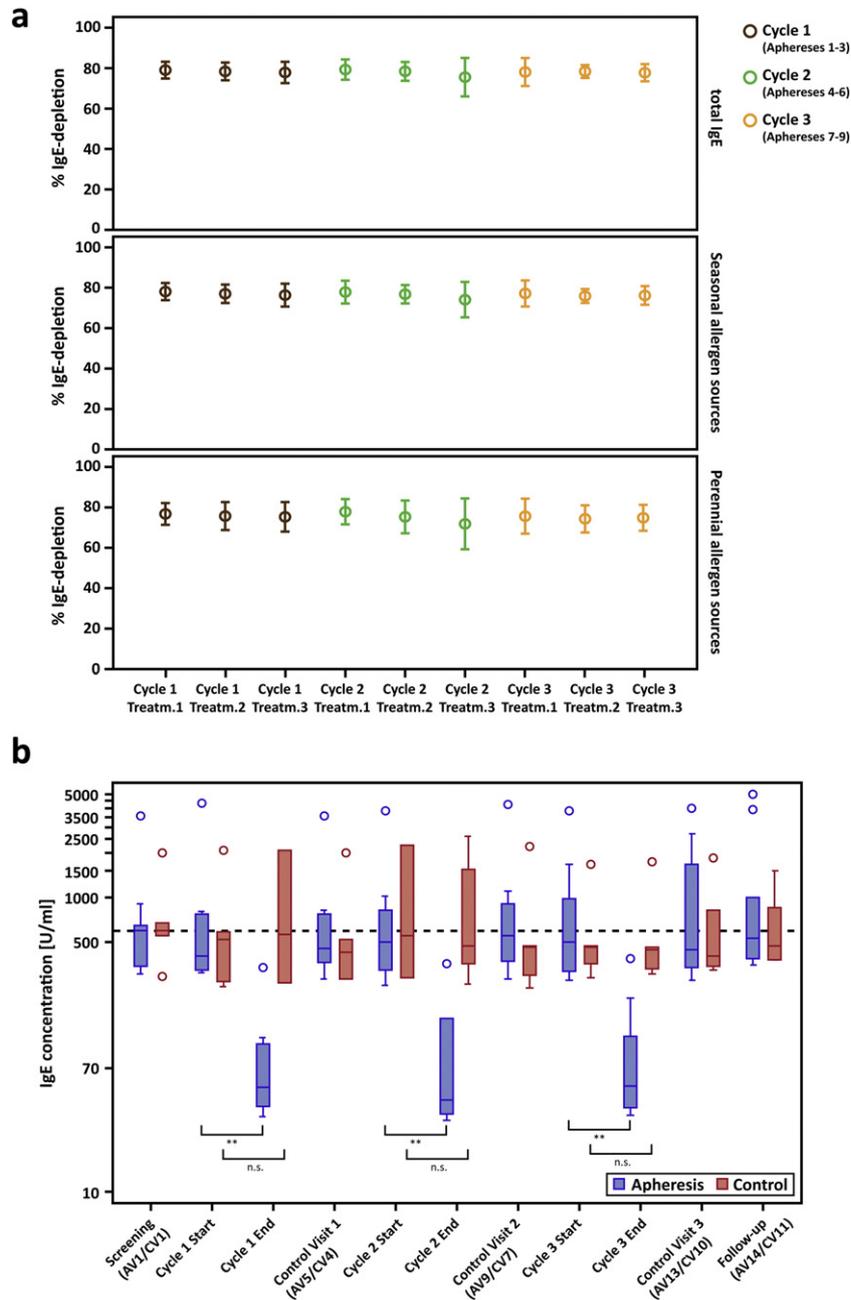


Fig. 2. Relative IgE-depletion and absolute IgE-levels. (a) Mean (\pm SD) depletion rates (y-axes: % IgE-reduction) of total IgE (top chart), IgE to seasonal (center) and perennial allergen sources (bottom) are shown for each treatment visit (x-axes) of the apheresis group. (b) Absolute total IgE-levels (y-axis: U/ml) are shown as box-and-whisker plots for the screening visit, the first (Start) and the last visit (End) of each treatment cycle, for control- and follow-up visits (x-axis). Blue boxes show results for the treatment, red boxes for the control group, outliers are indicated by circles. The dashed line corresponds to median total IgE-levels for both groups at the screening visit. Significant differences between start and end of the respective cycle are indicated (** $p \leq 0.01$).

306–4344 U/ml), IgE-depletion rates were similar in all patients (Table 4 and Table E1, online repository) and highly reproducible (observed differences between mean desorption rates of all 9 treatment days: $p = 0.95$). Comparing pre-treatment values of the very first apheresis (AV2pre; AV – apheresis visit) with post-treatment values of the last visit of cycle 3 (AV12post), the mean IgE-desorption rate was 86.2% ($\pm 5.1\%$ SD) in the intention-to-treat group. Thus, the primary endpoint of the study was achieved in all apheresis patients ($p < 0.0001$).

Reduction rates were almost identical for total IgE and IgE specific for seasonal and perennial allergen sources (Fig. 2a, Table E1, online repository). Significant reduction of absolute total IgE-levels between the first and the last visit of each cycle were observed in the apheresis group ($p = 0.004$) but not in the control group at corresponding time points.

At each control visit, i.e., 2 weeks after treatment cycles, IgE-levels had returned to baseline, indicating that IgE-depletion does not influence IgE-production (Fig. 2b, Table 4, Table E1, online repository).

Specificity of the treatment for IgE was demonstrated by comparing changes in IgG, IgA and IgM with changes in serum albumin (Fig. 3, Table 5).

3.3. Immunoabsorption with IgEnio is Safe and Well-tolerated

With the exception of 1 patient of the treatment group who withdrew informed consent for personal reasons before the first immunoabsorption treatment, all subjects completed the trial. All 9 patients of the intention-to-treat group were subjected to 3 complete

Table 4

Absolute levels and relative reduction of total IgE in the intention-to-treat population. Median, minimum (min.) and maximum (max.) total IgE-levels [U/ml] are shown for each visit for the apheresis (blue) and the control group (pink); pre – levels measured before apheresis; post – levels measured after apheresis; AV – visits of the apheresis group; CV – visits of the control group. Mean (\pm SD) relative reductions of total IgE-levels are shown for each treatment visit for the apheresis group. For both groups, mean (\pm SD) values of relative changes are shown for each treatment cycle or for corresponding weeks of the control group, respectively.

Visit	Apheresis group										Control group								
	total IgE [U/ml]				% change per treatment			% change per cycle ^a			total IgE [U/ml]				% change per cycle ^b				
	n	median	min.	max.	n	mean	SD	n	mean	SD	n	median	min.	max.	n	mean	SD		
AV1/CV1	9	592	304	3582							5	596	290	1987					
AV2pre/CV2	9	399	306	4344	9	79.0	4.2	9	87.6	3.4	5	514	246	2098	3	2.8	2.0		
AV2post	9	69	57.1	1067															
AV3pre	9	307	188	2393	9	78.4	4.4												
AV3post	9	51.6	42.6	486															
AV4pre	9	282	143	1487	9	77.9	5.3												
AV4post/CV3	9	51.9	32.3	333							3	557	261	2083					
AV5/CV4	9	453	278	3563							5	428	278	1991					
AV6pre/CV5	9	499	254	3871	9	79.3	5.0	9	86.1	3.9	3	549	285	2252	3	1.9	14.3		
AV6post	9	76.2	53.5	727															
AV7pre	8	367.5	206	2665	8	78.4	4.7												
AV7post	9	57.2	43.1	533															
AV8pre	9	294	132	1997	9	75.5	9.5												
AV8post/CV6	9	99.6	30.7	354							4	469	259	2579					
AV9/CV7	9	553	279	4278							5	456	241	2210					
AV10pre/CV8	9	501	274	3837	9	78.1	6.9	9	88.1	2.3	5	457	286	1684	5	0.6	5.8		
AV10post	9	73.9	50.5	803															
AV11pre	9	319	136	2439	9	78.4	3.2												
AV11post	9	56.2	29.7	571															
AV12pre	9	305	131	2120	9	77.7	4.2												
AV12post/CV9	9	52.9	33.4	384							5	439	305	1728					
AV13/CV10	8	443	275	3978							5	397	324	1836					
AV14/CV11	9	531	345	4971							5	472	375	1520					
All treatments/cycles					80	78.1	5.4	27	87.3	3.3					11	1.6	7.5		

^apre-value of the 1st vs. post-value of the 3rd treatment of the respective cycle;

^bvalue of the 1st vs. value of the 2nd visit of the same week

cycles of 3 aphereses each. All but one subject were treated per protocol (Fig. 1a). Due to low body weight of this patient (subject 009; Table 2) <80% of the planned plasma volume were desorbed during each apheresis.

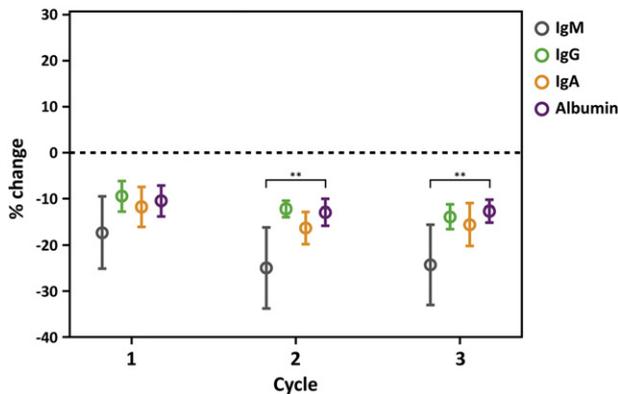


Fig. 3. Isotype specificity of IgEnio. Relative changes (y-axis: % changes to pre-treatment values) in serum IgM (gray), IgG (green), IgA (orange) and albumin (purple) are shown for each treatment cycle (x-axis) for the apheresis group. Error bars represent mean relative changes \pm standard deviations. Significant differences to changes in serum albumin are indicated (** $p \leq 0.01$).

Almost all adverse events (AE) judged by the investigators as treatment-related were clinically irrelevant deviations of laboratory parameters which are frequently observed after immunoadsorption, and resolved without any treatment within 1–2 days (not shown). Allergy-related AEs were mild skin-reactions and general disorders (Table 6).

During the complete study period, 7 severe adverse events (SAE) were recorded, 4 of which occurred during the treatment phase. Of these, 1 SAE was reported in the control group and 3 in the treatment group (Table 7). Only 2 SAEs emerged during apheresis treatments and were thus rated as treatment-associated: one patient reported chest pain that started while being treated, which turned out to be due to chronic degenerative changes of the spinal column. The second patient developed acute dyspnoea, starting a few minutes before completion of immunoadsorption. This acute condition resolved quickly under symptomatic medication and the patient could be discharged on the same day. This patient already had exhibited severe asthma attacks during the screening phase and was even hospitalized once due to an intractable status asthmaticus.

3.4. Immunoadsorption with IgEnio Reduces Sensitivity to Seasonal Pollen Allergens

During the study, patients were seasonally exposed to birch and grass pollen which is known to boost allergen-specific IgE-levels (Niederberger et al., 2007). We therefore analyzed birch- and grass pollen allergen-specific IgE-levels, as well as skin- and basophil-reactivity

Table 5

Mean relative changes (\pm SD) of serum IgM, IgG, IgA and albumin-levels are shown for each treatment day and -cycle. AV – visits of the apheresis group.

Visit	n	Apheresis group															
		IgM				IgG				IgA				Albumin			
		% change per treatment		% change per cycle ^a		% change per treatment		% change per cycle ^a		% change per treatment		% change per cycle ^a		% change per treatment		% change per cycle ^a	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
AV2	9	-15.2	11.5	-17.3	7.8	-8.9	3.2	-9.4	3.3	-10.5	6.3	-11.7	4.3	-10.5	2.0	-10.5	3.3
AV3	9	-22.2	13.4			-10.9	3.9			-14.7	6.4			-10.6	4.3		
AV4	9	-14.5	12.1			-8.6	6.9			-10.0	11.0			-10.3	6.1		
AV6	9	-21.2	6.7	-25.0	8.8	-12.1	3.0	-12.2	1.7	-14.0	3.8	-16.3	3.5	-15.0	6.8	-12.9	2.9
AV7	8	-24.8	9.9			-11.6	3.9			-17.0	3.7			-12.0	2.9		
AV8	9	-29.3	16.9			-13.1	5.1			-18.1	7.9			-12.1	3.2		
AV10	9	-25.1	14.4	-24.3	8.7	-15.5	4.2	-13.9	2.7	-15.6	9.2	-15.6	4.6	-13.8	4.7	-12.7	2.4
AV11	9	-24.7	4.6			-13.8	4.6			-15.2	4.3			-12.6	2.9		
AV12	9	-23.1	10.8			-12.4	2.9			-16.0	4.7			-11.6	3.3		

^a mean % changes of all 3 treatments of the respective cycle

in birch and grass pollen allergic subjects among our study participants. Patient 014 from the control group showed a strong boost of birch-specific IgE (Fig. 4c, left chart, purple curve) which was associated with an almost twofold increase in birch-specific basophil-reactivity. A more than fourfold increase in birch-specific skin-reactivity occurred with a delay of several weeks in this patient (Fig. 4a and b, left charts, purple squares). Birch pollen-specific IgE-levels, as well as basophil- and skin-reactivity of control patient 004 (dark green) showed no relevant changes during the birch season, while basophil-reactivity declined and no relevant change of skin-sensitivity to birch pollen was found. The strong increase in birch-specific IgE after week 10, i.e., outside the birch season, was due to polyclonal activation during a protracted respiratory infection, resulting in an increase of all IgE-specificities tested (Fig. E1, online repository). Unlike control patient 014, patients treated by apheresis (003, 005) showed no relevant increase of birch pollen-specific IgE-levels during the pollen season, there was a gradual decline in basophil-sensitivity and no increase in skin-sensitivity occurred in either patient (Fig. 4, left).

For the nine grass pollen allergic patients treated by apheresis, with the exception of patient 017, increases of grass pollen-specific IgE-levels were detected only at the follow-up visit (AV14) approximately four

weeks after the last apheresis (Fig. 4c, right). This is in contrast to the control group, where two out of three patients showed a boost already at control visit 8 (CV8, patient 013) or CV10 (patient 004), respectively, soon after start of the grass pollen season. Accordingly, we found that grass pollen-specific basophil-sensitivity in the apheresis group declined until AV13 and skin-sensitivity did not increase (Fig. 4a and b, right). Control patient 013, who had shown a more than 5-fold increase of grass pollen-specific IgE-levels also increased in basophil- and skin-sensitivity.

In contrast to seasonal allergen-sources, no significant differences were observed for perennial allergen sources (e.g., house dust mite) regarding allergen-specific IgE-levels, basophil- or skin-sensitivity (Fig. E2, online repository).

No effect of apheresis treatment on allergen-specific T cell proliferation was found (data not shown).

3.5. Treatment with IgEnio is Associated with Improved Lung Function and Reduced Lung Symptoms

Starting from similar baseline levels, a steady improvement in mean peak flow was observed in the apheresis group whereas the control group had relatively unchanged levels up to day 70 when, coinciding with the onset of the grass pollen season, a drop of peak flow levels was measured (Fig. 5a). In accordance with these data, apheresis patients showed continuous improvement regarding overall (VAS) and lung-specific symptoms while control subjects deteriorated in both parameters (Fig. 5b and c). No differences were found between the two groups for eye- and nose-related symptoms (not shown). Due to the small number of patients, results were not statistically significant.

Results from an Asthma Quality of Life Questionnaire (Juniper et al., 1999) were obtained from all but one patient (Table 8), showing a moderate change in symptoms in the apheresis group at the follow-up visit when compared to results from treatment start (n.s.). For activity limitation (n.s.), emotional function ($p = 0.02$) and total score (n.s.), minimal clinically important changes were observed. By contrast, no shifts in any parameter were recorded in the control group.

3.6. IgEnio Depletes IgE In Vitro in Presence of Omalizumab

It is known from previous studies (Jardieu and Fick, 1999) that in patients who are subjected to Omalizumab treatment, considerable concentrations of IgE/Omalizumab immune complexes accumulate in the blood. In a serum sample with moderately elevated total IgE-level (serum 1, 263 U/ml), addition of an equal amount of Omalizumab as related to the IgE-level did not interfere with IgE-depletion by IgEnio

Table 6

Mild allergy-related adverse events that occurred after the screening phase.

Symptom		Events	Apheresis	Control
Respiratory	Mild dyspnea	n	106	59
		n/patient	11.8	11.8
	Cough	n	85	29
		n/patient	9.4	5.8
	Wheezing	n	51	41
		n/patient	5.7	8.2
Pruritus	n	7	0	
	n/patient	0.8	0	
Skin	Urticaria	n	5	0
		n/patient	0.6	0
	Rash	n	4	0
		n/patient	0.4	0
	Angioedema	n	1	0
		n/patient	0.1	0

Table 7
Serious adverse events that occurred during the treatment phase.

Pat. Id.	Group	Serious adverse event (SAE)	Related to treatment	Intensity	Action	Outcome
003	Apheresis	Chest pain due to degeneration of intervertebral disc	Not related	Mild	Other	Partial remission of symptoms
004	Control	Dyspnea	Not related	Severe	Change of medication	Complete remission of symptoms
009	Apheresis	Acute dyspnea	Likely	Severe	Study treatment stopped, change of concomitant medication	Complete remission of symptoms
009	Apheresis	Patient fell under influence of alcohol	Not related	Mild	Other	Complete remission of symptoms

adsorber material *in vitro* (Fig. 6a and Table 9). Even in presence of a 40-fold excess of Omalizumab, *in vitro* depletion rates were only slightly lower than in the serum sample alone (76.8% versus 90% depletion). When the same experiment was performed using a serum sample with a higher total IgE-level, i.e., 1427 U/ml (serum 2), reduction of depletion rates from 87.1% in samples without Omalizumab to 55% was demonstrated, regardless if an equimolar amount or a 40-fold excess of Omalizumab were added (Fig. 6b and Table 9).

4. Discussion

Our study shows that IgE-desorption with IgEnio leads to selective and highly reproducible IgE-reduction rates of 87.3% per cycle in patients with a broad range of pre-treatment IgE-levels (306–4344 U/ml). This is in line with results from previous *in vitro* tests and indicates that subjects with even higher total IgE-levels can be treated with IgEnio. No relevant changes of IgE-levels were noted in the control group.

Since desorption with the single-use columns was done by peripheral venous access, treatment with IgEnio was convenient, safe and well-tolerated. In total, 81 treatments were performed by veno-venous access in highly sensitive patients suffering from allergic asthma and other allergic co-morbidities such as allergic rhinitis, atopic dermatitis and life-threatening food allergies. Most adverse events were subclinical deviations of laboratory parameters that resolved without any treatment. Mild respiratory AEs such as dyspnea, cough or wheezing occurred at similar frequencies in both groups. Skin reactions, such as pruritus, urticaria, rash and one case of angioedema were observed in the apheresis group, but rapidly resolved under antihistamines and steroids, respectively. Likewise, the only SAE likely related to the treatment, i.e., an asthma attack at the end of the first apheresis in one patient, resolved under medication and the patient was discharged on the same day. The remaining 8 treatments were well tolerated by this patient.

Even though aphereses required 3.5 h on average, compliance of patients was high, as indicated by the absence of drop-outs during the treatment phase.

Although the study was designed to investigate efficacy, specificity and safety of IgE-desorption by IgEnio, data on immunological and clinical effects were also collected. The trial was conducted from end of February until mid-July 2014, covering birch and grass flowering seasons. Usually, strong increases of allergen-specific IgE, basophil- and skin-sensitivity are observed in birch and grass pollen allergic patients after seasonal exposure (Niederberger et al., 2007). Interestingly, pollen allergen-specific basophil-sensitivity declined in the apheresis group

and skin-sensitivity did not increase after the pollen season. This may be explained by the fact that, due to removal of the newly synthesized IgE, cells were not loaded with the latter. Effects of IgE immunoabsorption on basophil-sensitivity appeared earlier than those on skin-sensitivity which may be due to the shorter life-span of basophils as compared to mast cells. Immunological effects observed were paralleled by clinical effects: improvement in peak flow was detected in the treated group already after the first cycle of immunoabsorption that was not reversed even during the peak of the birch pollen season (Fig. 5a). Likewise, steady improvement in lung symptoms was recorded for the apheresis group, in contrast to worsening in the control group after day 40, when the highest birch pollen counts were reported (Fig. 5c). After the onset of the grass pollen season, the 3rd cycle of immunoabsorption was performed and thus, all patients of the apheresis group had been subjected to two cycles already before being exposed to grass pollen. Shortly after start of the grass season, around day 70, the control group deteriorated in general and lung-associated symptoms and in peak flow measurements, whereas the treated group remained stable or even showed improvement (Fig. 5a–c).

A limitation of our study was that, due to the uneven distribution of allergic co-morbidities (i.e., rhinitis, dermatitis) in the study groups and due to the low number of study participants, effects of the treatment on co-morbidities could not be investigated. The effects of treatment with IgEnio on clinical endpoints will be assessed in follow-up studies. Likewise, effects of long-term treatment will be analyzed in future studies.

Based on these results, two treatment scenarios emerge: first, we suggest IgE immunoabsorption with IgEnio in patients with pollen-induced asthma attacks, initiated shortly before and continued during the pollen season. This treatment can be prescribed regardless of total IgE-levels due to the high binding-capacity of IgEnio. Since treatment with IgEnio was well tolerated, we think that it also can be applied in patients suffering from severe and uncontrolled asthma. Accordingly, the second clinical application suggested by our data concerns polysensitized allergic patients who are not eligible for Omalizumab treatment due to IgE-levels beyond the current limits of the drug (1500 U/ml in the EU (EMA, 2015) and 700 U/ml in the US (FDA, 2015)). Among our study population, 3 treated subjects had pre-treatment levels exceeding these limits (Table E1, online repository, patient 002: 4344 U/ml, patient 006: 797 U/ml, patient 017: 764 U/ml). In such patients, IgE-levels could be reduced by immunoabsorption in a first step, followed by administration of Omalizumab. An equivalent strategy already was successfully applied in a child suffering from severe asthma (Kerzel et al., 2011), in patients with severe atopic dermatitis (Zink et al., 2016) and in a case of life-threatening food allergy (Dahdah et al., 2015). However, reduction of total IgE-levels in these patients was

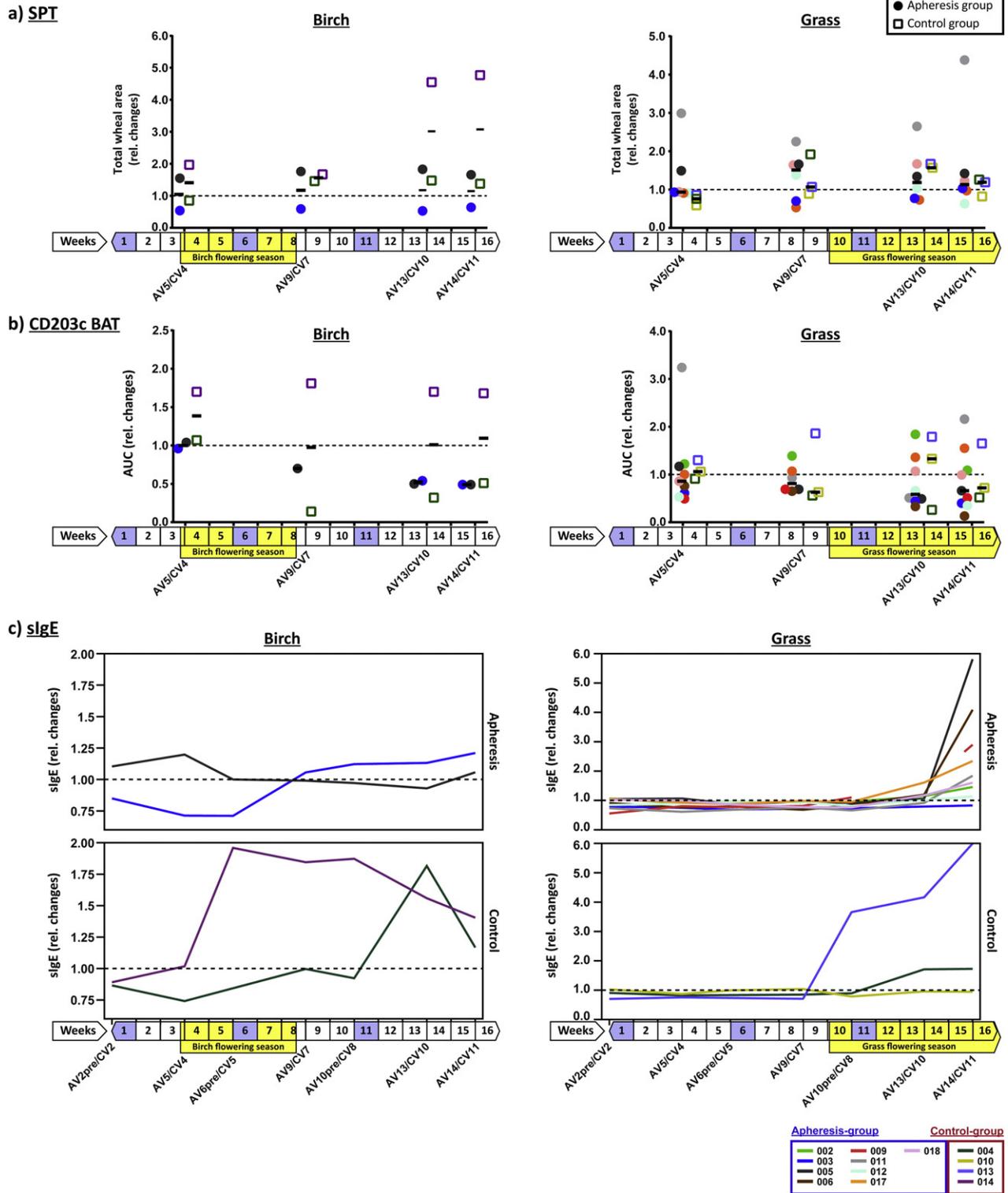


Fig. 4. Changes in allergen-specific skin- and basophil-reactivity and in sIgE-levels. Relative changes (y-axes: fold changes) of (a) total wheal-areas from skin prick testing (SPT) and (b) of basophil-sensitivity (CD203c BAT – basophil activation test measuring CD203c upregulation; AUC – area under the curve) are shown as scatter plots for control and follow-up visits (x-axes) for birch (left) and grass pollen allergens (right). Results from individual subjects are colour-coded and displayed for the apheresis (filled circles) and for the control group (open boxes) with the respective median levels indicated by horizontal black lines for each group and visit. (c) Relative changes of sIgE-levels to birch (left) and grass (right) are displayed for the same subjects from (a) and (b) (top-charts: apheresis group, bottom-charts: control group). Weeks of study duration are shown underneath the x-axes with birch- and grass seasons highlighted by yellow boxes and treatment weeks in purple. Dashed horizontal lines indicate baseline levels. AV – visit apheresis group; CV – visit control group.

achieved either by plasma exchange (Kerzel et al., 2011), a very cumbersome and unspecific treatment, by using an adsorber non-specifically removing antibodies of all isotypes (Zink et al., 2016) or by an IgE-

specific adsorber that also reduced IgG, IgM and IgA by approximately 40% (Dahdah et al., 2015). In contrast, IgE-depletion with IgEnio would facilitate Omalizumab administration after fewer desorptions

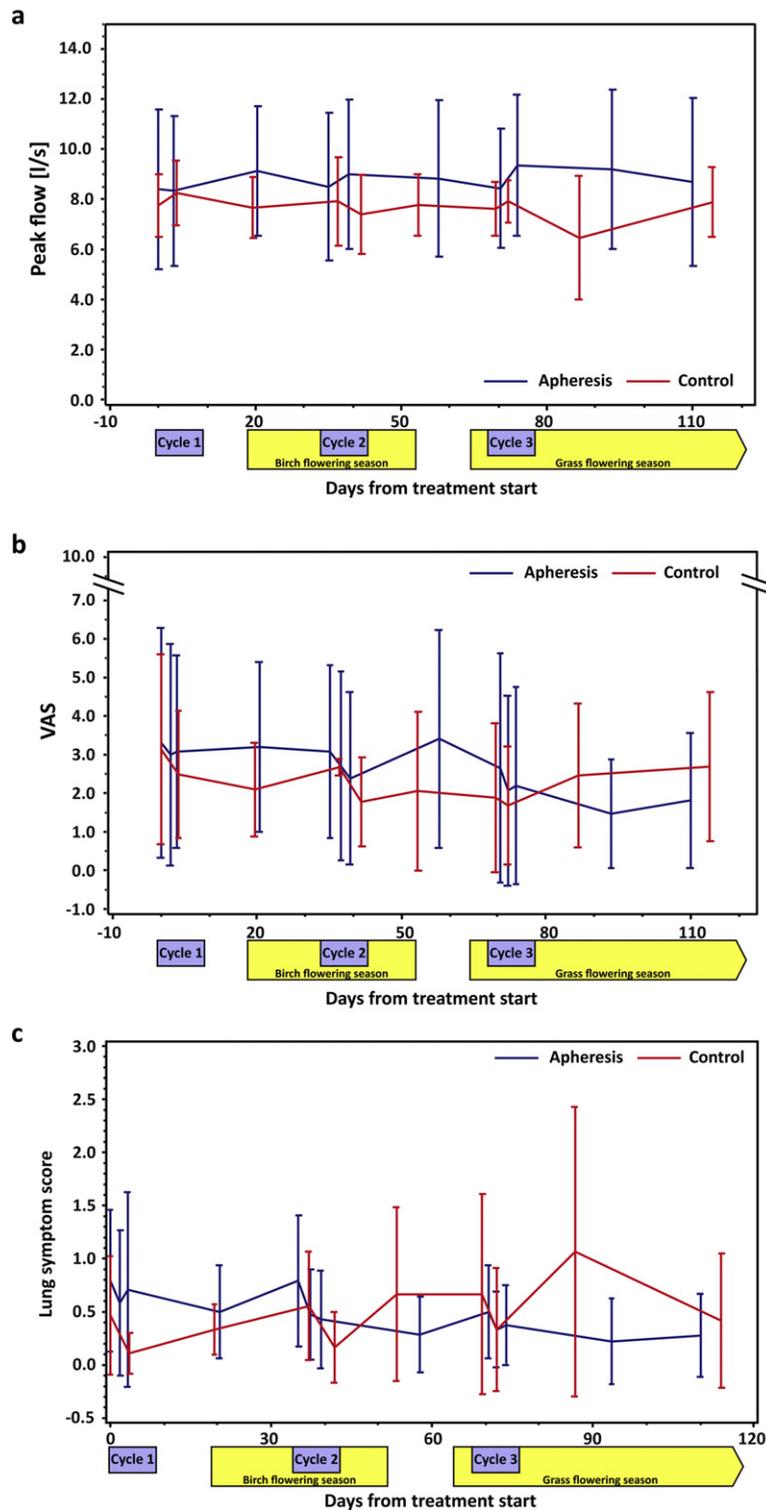


Fig. 5. Changes in peak flow and symptom scores. (a) Peak flow results (y-axis: l/s), (b) overall severity of allergy-related symptoms (y-axis: VAS – visual analogue scale) and (c) severity of lung symptoms (y-axis: lung symptom score), based on daily assessment by the patients, are shown for the complete study period (x-axes: days from treatment start). Blue curves represent results from the apheresis group and red curves from the control group. Error bars show mean values, \pm SD. Treatment weeks are highlighted by purple boxes, birch and grass pollen seasons by yellow boxes.

and without alteration of other plasma components than IgE which would allow to use IgEnio also in children.

In addition, results from *in vitro* experiments demonstrated that IgEnio also depletes IgE in presence of Omalizumab (Fig. 6, Table 9). This observation indicates that IgEnio may also be used in Omalizumab-treated patients with extremely elevated IgE-levels in order to remove

potentially harmful immune complexes. Other treatment scenarios to be studied include application in polysensitized patients, in atopic dermatitis (Kasperkiewicz et al., 2011, 2014; Reich et al., 2016) and in the context of allergen-specific immunotherapy.

In summary, we provide evidence that IgE immunoadsorption with IgEnio is a treatment strategy for patients with severe pollen-induced

Table 8

Results from a validated Asthma Quality of Life Questionnaire. In every category, several questions had to be answered by selection of a value between 1 (complete impairment) and 7 (no impairment). Differences between results obtained at the end and at the beginning of the trial are shown. SD – standard deviation; min – minimum; med – median; max – maximum.

Parameters	Group	n	mean	SD	min	med	max
Total score	Apheresis	8	0.84	1.26	-1.6	0.8	2.7
	Control	5	0.14	0.28	-0.3	0.3	0.4
Symptoms	Apheresis	8	1.02	1.93	-2.7	1.1	3.6
	Control	5	0.10	0.15	0.0	0.0	0.3
Activity limitation	Apheresis	8	0.90	1.08	-0.9	0.8	3.0
	Control	5	0.16	0.42	-0.5	0.3	0.5
Emotional function	Apheresis	8	0.80	0.76	-0.2	1.1	1.8
	Control	5	0.12	0.44	-0.4	0.0	0.8
Environmental stimuli	Apheresis	8	0.26	1.25	-2.3	0.5	1.8
	Control	5	0.20	0.78	-0.5	0.0	1.5

Clinical significance:
 Minimal important difference: ≥ 0.5 , < 1
 Moderate change: ≥ 1 , < 1.5
 Large change: ≥ 1.5

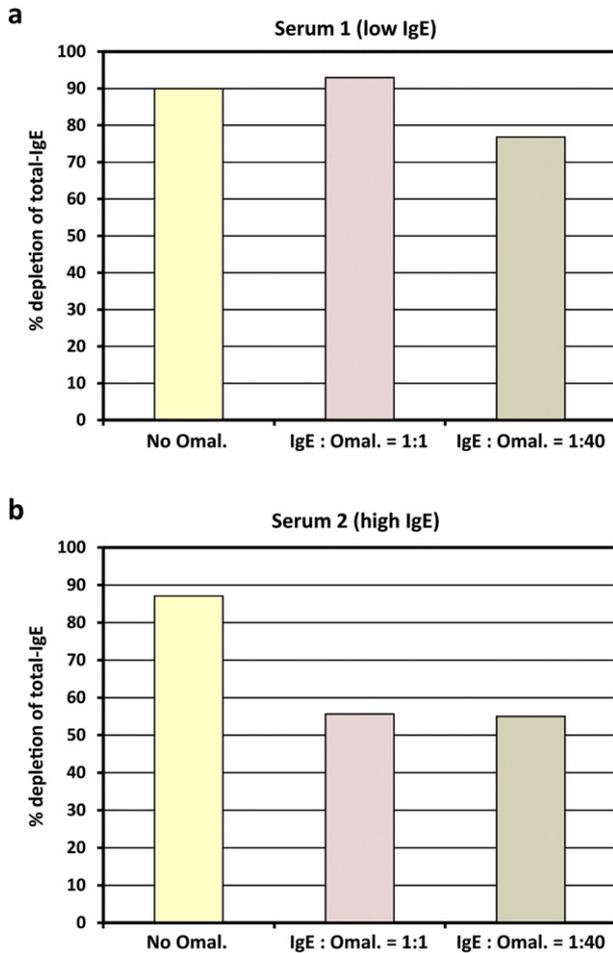


Fig. 6. *In vitro* IgE-depletion from serum samples after addition of Omalizumab. Two serum samples with total IgE-levels < 300 U/ml (a, low IgE) or > 1400 U/ml (b, high IgE), without (yellow bars) and with different IgE-to-Omalizumab ratios (x-axes: 1:1 – pink bars; 1:40 – brown bars) were applied to IgEnio adsorber material. Relative depletion rates of total-IgE are shown (y-axes).

asthma attacks and for allergic patients with high serum total IgE-levels. Additional clinical investigations with a higher number of participants are planned to confirm these results and to further improve treatment efficacy.

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Table 9
In vitro depletion of IgE from sera after addition of Omalizumab.

Sample	total-IgE [U/ml]	% IgE-depletion
Serum untreated	263	-
Serum 1 (low IgE)		
No Omal. pre	268	0.0
No Omal. post	27	90.0
IgE : Omal. = 1:1 pre	247	0.0
IgE : Omal. = 1:1 post	17	93.0
IgE : Omal. = 1:40 pre	272	0.0
IgE : Omal. = 1:40 post	63	76.8
Serum 2 (high IgE)		
Serum untreated	1427	-
No Omal. pre	1669	0.0
No Omal. post	215	87.1
IgE : Omal. = 1:1 pre	1436	0.0
IgE : Omal. = 1:1 post	637	55.6
IgE : Omal. = 1:40 pre	1466	0.0
IgE : Omal. = 1:40 post	659	55.0

Omali. – Omalizumab; pre – samples obtained before application to IgEnio; post – samples obtained after application to IgEnio.

Conflicts of Interest

Christian Lupinek's salary during the conduction of the trial was partly funded by Fresenius Medical Care (Bad Homburg, Germany).

Rudolf Valenta has received research grants from Biomay AG (Vienna, Austria), Thermo Fisher (Uppsala, Sweden) and Fresenius Medical Care (Bad Homburg, Germany) and serves as consultant for these companies.

René Cervenka, Thomas Plaichner, Hans Peter Leinenbach, Adelheid Gauly and Ingrid Uhlenbusch-Koerwer are employees of Fresenius Medical Care (producer of IgEnio and sponsor of the study).

Volker Schoder and Justyna Kozik-Jaromin were employees of Fresenius Medical Care (producer of IgEnio and sponsor of the study) when the trial was planned and conducted.

Gottfried Stegellner, Hans Huber and Rainer Henning are employees of Biomay AG (producer of BM10, the ligand of IgEnio).

No conflicts of interest related to the present article: Kurt Derfler, Silvia Lee, Thomas Prikoszovich, Oliver Movadat, Eva Wollmann, Ventsislav Petkov, Carolin Cornelius, Milena Weber, Renate Fröschl, Regina Selb, Katharina Blatt, Dubravka Smiljkovic, Thomas Perkmann, Verena Niederberger, Peter Valent.

Author Contributions

Christian Lupinek, Rudolf Valenta: study design, data analysis and interpretation, paper writing, figures.

Kurt Derfler, Silvia Lee, Thomas Prikoszovich, Oliver Movadat, Eva Wollmann, Ventsislav Petkov: recruitment, treatment and clinical examination of patients, critical reading of the paper.

Carolin Cornelius, Milena Weber, Renate Fröschl, Regina Selb, Katharina Blatt, Dubravka Smiljkovic, Thomas Perkmann, Verena Niederberger, Peter Valent: immunological and serological *in vitro* testing, critical reading of the paper.

Volker Schoder: sample size calculation, planning and execution of statistical data analysis.

René Cervenka, Thomas Plaichner, Hans Peter Leinenbach: development (coupling of the ligand, sterilization) and pre-clinical testing of IgEnio, production of columns for the clinical trial, critical reading of the paper.

Gottfried Stegellner, Hans Huber, Rainer Henning: process development and production of BM10, i.e., the ligand for IgEnio.

Justyna Kozik-Jaromin: safety and SAE reporting during the trial, critical reading of the paper.

Adelheid Gauly, Ingrid Uhlenbusch-Koerwer: study design, coordination of the study team, critical reading of the paper.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ebiom.2017.02.007>.

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