Study protocol

Extracorporeal **SP**ecific **I**gE **R**emoval from the plasma of allergic **A**sthma patients (ESPIRA-study)

Study code:	TA-IgE-01-A
Study centre:	Medical University Vienna
Investigational device:	Single use IgE adsorber
Control device:	not applicable
Study design:	Prospective, single-centric, open, controlled, randomized, comparative, interventional
Protocol status:	Final Version 1.0
Date of current version:	2013-07-30

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RESPONSIBILITIES, SIGNATURE PAGE

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Study Protocol: **E**xtracorporeal **SP**ecific **I**gE **R**emoval from the plasma of allergic **A**sthma patients (ESPIRA-study)

Protocol status: Final Version 1.0, July 30, 2013

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Study Protocol: Extracorporeal SPecific IgE Removal from the plasma of allergic Asthma patients (ESPIRA-study)

Protocol status: Final Version 1.0, July 30, 2013

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STUDY SYNOPSIS

Title:	**E**xtracorporeal **SP**ecific **I**gE **R**emoval from the plasma of allergic **A**sthma patients (ESPIRA-study)
Study code:	**TA-IgE-01-A**
Study design:	Prospective, single-centric, open, controlled, randomized, comparative, interventional
Investigational device:	Single use IgE adsorber
Control:	Standard drug treatment
Study objectives:	*Primary objectives* • To determine if the new IgE adsorber is able to reduce IgE levels • To evaluate if the new IgE adsorber can be safely used in the clinic *Further assessments* • To determine if IgE reduction induced by the IgE adsorber results in changes of immunological and clinical parameters • To demonstrate that no IgE reduction by at least 50% can be observed in the control group without apheresis therapy.
Study hypotheses:	The new IgE adsorber is capable of reducing IgE in plasma/serum by at least 50% measured before the first treatment in the first cycle and after the last treatment in the last treatment cycle
Patients:	15 allergic asthmatic patients: 10 patients treated with the new IgE adsorber and conventional treatment, 5 patients as control with conventional treatment only. The control group provides a group of patients for the assessment of natural fluctuations of IgE levels as well as for medication intake and symptoms.
Inclusion criteria:	*General* • 18 to 50 years old • Male and female (contraception during study participation) • Signed Informed Consent

Study-specific

- Clinically relevant allergy to respiratory allergens confirmed by patient's history, skin-prick testing, IgE serology
- Allergic asthma (diagnosed by lung specialist)
- IgE concentration in blood at least 300 kU/L

Exclusion criteria:

General

- Participation in another trial within 30 days prior to enrolment
- Gravidity (pregnancy test prior to each treatment cycle)

Study-Specific

- Intake of omalizumab
- Hepatitis or HIV or malignant disease
- Specific immunotherapy in the last 6 months
- Non-allergic asthma
- Auto-immune diseases
- Hypocalcaemia
- Intake of ACE-inhibitors (discontinuation according to half-time before treatment possible)
- Uncontrolled bleeding and coagulation disorders
- Severe cardiovascular disease
- Severe cardiac arrhythmias
- Severe systemic infection
- Unability to tolerate therapeutic apheresis procedures (hypersensitivity associated with previous apheresis sessions)
- Inadequate peripheral venous access
- Condition in which acute fluid shifts may cause congestive heart failure
- Established or suspected intra-cranial disease where fluid imbalance or pressure changes could exacerbate the disease
- Impaired renal function
- Clinically significant hypotension or borderline hypotension where a further drop in blood pressure could be harmful

Treatment:

Patients randomized to apheresis will be treated 3 times within a period of 5 to 12 days (one treatment cycle) with apheresis using

the new IgE adsorber. During each visit 2 plasma volumes will be processed at a maximum blood flow rate of 120 ml/min and at a maximum plasma flow of 30 ml/min. A second treatment cycle is performed after a 4 to 5 weeks interval, and a third cycle 4 to 5 weeks thereafter. Patients randomized to the control group will receive conventional drug treatment only like patients in the apheresis group as prescribed before study entry.

Target variables:

Primary variable:

- Relative reduction of IgE after the last treatment cycle compared to pre-treatment levels of the first treatment in the first cycle

Safety parameters:

- Serious adverse events
- Adverse events
- Electrolyte balance (sodium, potassium, total calcium, ionized calcium, chloride, phosphate)
- Thrombogenicity (aPTT, ionized calcium, TAT, factor VIII, fibrinogen)
- Complement activation (C3a, C5a)
- Liver function (alkaline phosphatase CK, LDH, AST, ALT, G-GT, total bilirubin)
- Kidney function (urea/BUN, uric acid, creatinine)
- Urine analysis
- Blood count: erythrocytes, thrombocytes, leukocytes, differential: MCV, MCHC, MCH), haemoglobin, haematocrit
- Vital signs (heart rate, blood pressure, temperature)
- Blood gas analysis parameter (blood oxygen saturation pH, oxygen partial pressure, carbon dioxide partial pressure, bicarbonate and Base Excess)
- ECG
- C-Reactive Protein (CRP)
- Total protein, albumin
- Lipids (cholesterol, LDL, HDL, triglycerides)
- Glucose

Other parameters:

- Cutaneous allergen-specific sensitivity determined by the skin prick test
- Allergen-specific IgE and IgG reactivity profile

- Reduction of IgE over the course of one treatment
- Specific IgE-levels considering the patient's individual sensitization profile
- IgE-positive cells in PBMCs
- FcEpsilonRI-expression on PBMCs
- Allergen-induced T cell activation and cytokine secretion
- Allergen-induced basophil activation
- CD203c-expression on PBMCs
- Medication
- Lung function (Peak Expiratory Flow, Forced Expiratory Volume)
- Asthma Control Questionnaire

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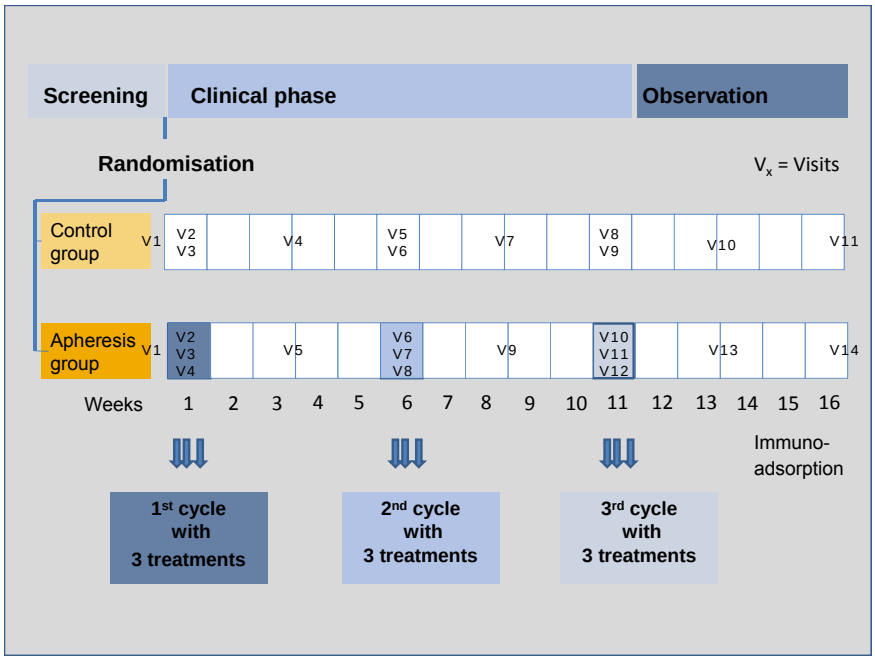
Planned start and end of study: October 2013 until April 2014

Clinical study plan:



Study schedule (Treatment 1 to 3 reflect 1 cycle which is repeated after 4-5 weeks, and for the third time after 4-5 more weeks)

	Screening and inclusion	Apheresis treatment 1, 4, 7		Apheresis treatment 2, 5,8	Apheresis treatment 3, 6, 9		Period between cycles		At the end of study
Visit number control group (CVx) and apheresis group (AVx) according to the above overview	CV1, AV1	CV2, CV5, CV8	AV2, AV6, AV10	AV3, AV7, AV11	CV3, CV6, CV9	AV4, AV8, AV12	CV4, CV7, CV10	AV5, AV9, AV13	CV11, AV14
Informed consent	X								
In-/Exclusion Criteria	X								
Year of birth	X								
Gender	X								
Body height and weight	X								
Medical history	X								
Medication (including data in Patient Diary)	X								X
Pregnancy test	X	X	X				X (CV10)	X (AV13)	
Lung function tests (PEF, FEV), physical examination	X						X	X	X
Asthma Control Questionnaire	X								X
Prick test	X						X	X	X
IgE and IgG reactivity profile (microarray test)	X								X
IgE concentration	X	X	X pp	X pp	X	X pp	X	X	X
Specific IgE-levels (acc. to individual profile)	X	X	X pp	X pp	X	X pp	X	X	X
IgE positive cells in PBMCs	X						X	X	X
FcEpsilonRI-expression on PBMCs	X						X	X	X
Allergen-induced T cell activation and cytokine secretion	X						X	X	X
Allergen-induced basophil activation	X						X	X	X
CD203c-expression on PBMCs	X						X	X	X
Safety parameters: CRP* (only pre), fibrinogen, factor VIII	X	X	X pp	X pp	X	X pp	X	X	X
Urine analysis	X	X	X	X	X	X	X	X	X
Temperature			X pp	X pp		X pp			
ECG	X		X pre			X post			
Clinical chemistry (see below)	X	X	X pp	X pp	X	X pp	X	X	X
Haematology (see below)	X	X	X pp	X pp	X	X pp	X	X	X
Blood pressure (every 60 minutes during treatment)	X		X	X		X			
Heart rate (continuously during treatment)	X		X	X		X			
Treatment time (start/stop)			X	X		X			
Blood flow/plasma flow			X	X		X			
Biocompatibility (TAT, C5a, C3a)			X pp	X pp		X pp			

Bedside tests (aPTT, ionized calcium, Blood Gas Analysis)			X	X		X			
Adverse and serious adverse events	X	X	X	X	X	X	X	X	X

X pp = sample pre and post apheresis;

*** CRP is measured only pre-treatment**

Clinical chemistry: sodium, potassium, total + ionized calcium, chloride, phosphate, alkaline phosphatase, CK, LDH, AST, ALT, G-GT, total bilirubin, urea/BUN, uric acid, creatinine, glucose, cholesterol, LDL, HDL, triglycerides, total protein, albumin,

Haematology: Erythrocytes, thrombocytes, leukocytes (neutrophils, basophils, eosinophils, lymphocytes), differential: MCV, MCHC, MCH, haemoglobin, haematocrit,

PEF: Peak Expiratory Flow; **FEV:** Forced Expiratory Volume

Urine analysis: urea/BUN, uric acid, creatinine, bilirubin, specific gravity, pH, leukocyte value, urobilinogen, protein ketone bodies, nitrite, glucose, erythrocytes

Blood Gas Analysis (BGA): pH, oxygen partial pressure pO_2, carbon dioxide partial pressure pCO_2 , bicarbonate HCO_3 (act and std) and Base Excess (BE)

Blue coulour: Tests performed at the Pulmonology Department

Grey coulour: Tests performed at the Immunology Department

White coulour: Tests perfomed at the Dialysis and Transplantation Department

ABBREVIATIONS

List of all abbreviations used in the clinical study protocol and in the Case Report Forms in alphabetical order

ACE	Angiotensin converting enzyme
ACQ	Asthma Control Questionnaire
ADE	Adverse device effect
AE	Adverse event
ALT	Alanin-Aminotransferase
aPTT	activated Partial Thromboplastin Time
ASADE	Anticipated serious adverse device effect
AST	Aspartat-Aminotransferase
BGA	Blood gas analysis
BUN	Blood urea nitrogen
CE	Communauté Européenne, conformity according to 93/42 ECC XII
CIP	Clinical investigation plan (= clinical study protocol)
CK	Creatinine kinase
CRF	Case Report Form
CRP	C-reactive protein
CRO	Contract research organisation
EC	Ethics committee
ECG	Electrocardiogram
EEC or EC	European (economic) community
FAS	Full analysis set
FEV	Forced Expiratory Volume
FME	Fresenius Medical Care Deutschland GmbH
GCP	Good clinical practice guideline (for medical devices in ISO 14 155)
GGT	Gamma-Glutamyl-Transferase
HDL	High density lipoprotein
IgE	Immunglobuline E
IVD	*In vitro* diagnostic
ISO	International organization for standardization
ITT	Intention-to-treat population
LDH	Lactat dehydrogenase
LDL	Low density lipoprotein
MDD	(European) medical device directive
MEDDEV	Medical device guidelines of European commission
MedDRA	Medical dictionary for regulatory activities
MPG	Medizinprodukte Gesetz (German and Austrian medical device act)
PBMC	Peripheral Blood Mononuclear Cells
PEF	Peak Expiratory Flow
PP	Per-protocol-population
RR	Reduction rate
SADE	Serious adverse device effect

SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SMB	Safety Monitoring Board
SP	Safety-population
TAT	Thrombin Anti Thrombin
USADE	Unanticipated serious adverse device effect
WHO-ATC	Anatomical therapeutic chemical classification system of WHO
WHO	World health organization

1. INTRODUCTION

IgE-associated allergy affects more than 25% of the world population. The symptoms of IgE-associated allergy include mild forms such as hay fever and dermatitis but also severe and life-threatening manifestations such as asthma and anaphylactic shock. For mild forms of allergy and patients who are sensitized against few allergen sources there are several therapy options such as pharmacotherapy with antihistamines and allergen-specific immunotherapy. However, the majority of allergic patients are sensitized against multiple allergen sources and patients suffering from asthma need a battery of pharmacological treatments and are often uncontrolled despite taking combinations of bronchodilators and high doses of corticosteroids.

Immediate and also late phase allergic inflammation are mediated by IgE antibodies. Regarding immediate allergic inflammation, IgE-antibodies mediate the release of preformed pro-inflammatory mediators, cytokines and proteases by mast cells and basophils upon contact with allergen molecules. It is well established that serum levels of total IgE positively correlate with the density of the high-affinity IgE-receptor, FcEpsilonRI, expressed on the surface of these cell types (1). Therefore, high serum IgE levels cause increased sensitivity of mast cells and basophils leading to increased clinical symptoms of immediate allergic inflammation.

Besides mediating acute allergic inflammation, IgE antibodies are also important for late phase and chronic allergic inflammation. In fact, IgE antibodies bound to the high affinity and low affinity receptors on antigen presenting cells such as B cells, monocytes and dendritic cells have been shown to facilitate allergen-presentation to T cells, a process termed IgE-facilitated allergen-presentation (2). Due to its central role in the pathophysiology of allergic diseases, IgE is a major target for the development of new therapeutic approaches for IgE-associated allergy.

Several years ago, one of the most innovative drugs, based on an injected anti-IgE antibody has been successfully introduced into the clinics for the treatment of allergic asthma (3). This humanised monoclonal anti-IgE antibody, termed omalizumab, is administered subcutaneously and is thought to act by complexing unbound IgE in the blood and other compartments preventing IgE from combining with its receptors (3). Omalizumab has been shown to reduce asthma exacerbations significantly and spares steroids. However, the drug has to be titrated according to plasma IgE levels, and is confined to patients below an upper limit of 700 kU/l of plasma IgE in the USA and 1500 kU/l in Europe. Therefore, a considerable number of asthmatic patients cannot benefit from the treatment. Furthermore, it

was found that omalizumab induces in approximately 0.2% of patients anaphylactic reactions. For this reason it carries a boxed warning on the package insert.

Moreover, allergic individuals under treatment with omalizumab, show an increase of total serum IgE-levels, i.e., of free IgE and of IgE in complex with omalizumab (4). This observation maybe explained by cross-linking of the membrane-associated form of IgE on IgE-producing B-cells.

Other unwanted effects of omalizumab include the possible deposition of IgE/anti-IgE complexes during long-term treatment, anaphylactic reactions as well as an increased risk for arterial thrombosis (5,6).

In order to overcome these limitations and potential risks of injected anti-IgE treatment, we would like to adopt the well-established and safe immunoadsorption treatment which for decades has been used for safe and successful therapy of auto-immune disorders such as Myasthenia Gravis, systemic Lupus Erythematodes for the treatment of allergic asthma (7). In fact, there are first studies demonstrating that plasmapheresis and immunoadsorption may be effective for the treatment of patients suffering from severe forms of allergy (8,9). For this purpose, an IgE adsorber was developed which specifically binds IgE antibodies in human body fluids (10,11). The major expected advantages of this strategy to deplete IgE antibodies are:

- No limitations regarding IgE-levels to be treated. Also patients with IgE levels >700 kU/L and even >1500 kU/L can be treated.
- No administration of foreign protein into the patient.
- No generation of immune complexes bearing the risk of deposition in various organs.
- No systemic side effects as observed in the course of omalizumab treatment.

The ligand used for the production of the IgE adsorber is a single chain antibody fragment (scFv) with high affinity for IgE which does not cross-link effector cell bound IgE and therefore is not anaphylactogenic (11). The scFv is produced in high purity, free of mammalian proteins using an *Escherichia coli*-based expression system under the most stringently controlled conditions (i.e. GMP production). In the proposed trial, ten patients with documented IgE-associated allergic asthma will undergo IgE immunoapheresis (i.e. immunoadsorption) with the IgE adsorber in order to study the efficacy of the device to reduce systemic IgE levels in the patients and to assess the safety of this treatment. If the treatment proves to be effective in removing IgE, is safe and well tolerated, further studies are planned to develop an IgE-apheresis-based treatment for asthmatic patients who are currently insufficiently treated by the available medication.

2. OBJECTIVES OF THE STUDY

2.1. STUDY OBJECTIVES

The major objectives of this study are to demonstrate that IgE immunoapheresis based on the newly developed IgE adsorber is safe and well tolerated and can reduce IgE serum levels by more than 50% over all applied treatments whereas no such reduction can be observed in a matched control group with pharmacotherapy alone.

2.2. STUDY HYPOTHESIS

The study hypothesis is that the newly developed IgE adsorber will selectively deplete IgE antibodies from the blood of treated patients since the scFv is highly specific for IgE antibodies. It is expected that IgE levels in plasma/serum are reduced by at least 50% measured before the first treatment in the first cycle to the post-treatment value measured after the last treatment in the last treatment cycle.

2.3. TARGET VARIABLES

2.3.1 Primary variables

Reduction of IgE is the primary variable as it is demonstrated that diminishing IgE in allergic asthmatic patients improves clinical outcome (12). Thus elimination of IgE by the IgE adsorber is the main efficacy parameter.

2.3.2 Secondary variables- safety parameters

Serious adverse events and adverse events (definitions for both under 7.1) are recorded during the study in a manner which is described in chapter 7.
The following safety parameters will be analysed by the laboratory, unless indicated otherwise:

Electrolytes and Blood Gas Analysis

The concentrations of sodium, potassium, total calcium, phosphate and chloride will be analysed in the blood before and after treatment. Capillary blood gas analysis is performed bedside for estimation of disturbances in the acid base balance and the oxygen saturation of the blood.

Coagulation

The aPTT and ionized calcium will be determined bedside to control anticoagulation with heparin and citrate, respectively. Thrombin Anti Thrombin III (TAT) complex is measured to detect any activation of the coagulation cascade. Fibrinogen is adsorbed to some immunoadsorption devices and therefore, its concentration will be analysed before and after

treatment. Factor VIII can also be adsorbed to immunoadsorption devices and will, therefore, be analysed pre/post treatment.

Complement activation

As marker for complement activation, the levels of C3a and C5a will be analysed.

Liver and kidney function

For monitoring of liver and kidney function AST, ALT, G-GT, creatinine, bilirubin, uric acid and urea/BUN will be analysed. A special urine analysis will be performed with determination of specific gravity, pH, leukocyte value, bilirubin, urobilinogen, protein ketone bodies, nitrite, glucose, erythrocyte counts in order to control kidney function. Changes in lipids (cholesterol, LDL, HDL, triglycerides) are also recorded.

Additional safety markers from blood

Alkaline phosphatase will be used as a marker for bone metabolism and lactate dehydrogenase in order to monitor haemolysis/general cell lysis. Furthermore, albumin will serve as indicator for protein loss and dilution effects. CRP is a marker for infection.

Blood count

White blood cells (neutrophils, basophils, eiosinophils, lymphocytes), red blood cells and differential (MCV, MCHC, MCH), platelets, haemoglobin, and haematocrit will be analysed to determine any changes to the blood cell composition.

Injury of heart and other muscles

Creatine phosphokinase (CK or CPK) is measured as marker for muscle injury, especially of the heart. An electrocardiogram (ECG) is taken; pulse and blood pressure are determined by the site staff to confirm the patient's general condition.

Vital signs

Measurement of the temperature provides an indication for infection.

2.3.3 Further parameters

Treatment time

The treatment time will be calculated as the difference between stop and start time of the individual treatments.

Processed plasma volume

This parameter serves to correlate the volume of plasma treated to the change of plasma parameters.

Blood flow/plasma flow

Both parameters influence the performance of the IgE adsorber and are therefore monitored.

Immediate cutaneous sensitivity determined by prick testing

Titrated skin prick tests with allergen extracts prepared from up to two respiratory allergen sources according to the patients sensitization profiles will be performed at visits 1, 5, 9, 13 and 14 in the apheresis group and at visits 1, 4, 7, 10 and 11 of the control group. For this purpose registered and standardized skin prick test solutions from Stallergenes (Antony, France) will be diluted in 1:2 steps to determine the threshold concentration necessary to elicit a positive skin reaction (13). As positive and negative control, histamine hydrochloride and sodium chloride solutions (Stallergenes), respectively will be tested. The mean wheal area will be determined after 15 minutes by surrounding with a ball point pen and after transfer to paper by scotch tape will be subjected to digital planimetry (14, 15).Both, changes in thresholds concentrations required for eliciting a positive skin reaction as well as changes in wheal areas provide an objective measurement for a reduction of allergen-specific clinical sensitivity of the patients after treatment.

ASSESSMENT OF IMMUNOLOGICAL PARAMETERS

Allergen-specific IgE and IgG reactivity profiles/levels

Total and allergen-specific IgE levels will be measured in serum and/or plasma samples using quantitative ImmunoCAP (Phadia/Thermofisher, Uppsala, Sweden) measurements (10). In addition, IgE and IgG reactivity profiles will be determined using ISAC immunoCAP tests based on micro-arrayed allergens as described (16). Quantitative measurement of total IgE will allow to determine the extent of IgE depletion by the IgE adsorber. The parallel measurement of allergen-specific IgE and IgG levels will allow to determine the selective depletion of allergen-specific IgE.

Cellular assays (FcEpsilonRI-expression on basophils)

The intensity of expression of the high affinity receptor for IgE on basophils and antigen presenting cells will be determined by FACS analysis as described elsewhere (17). The intensity of expression of the low affinity receptor for IgE by various cell populations in patients such as PBMCs and in particular B cells, will also be determined by FACS analysis as described (18). Likewise, IgE-positive cells will be analysed by FACS analysis as decribed (17, 11). Assessment of the effects of IgE depletion by IgE adsorption on the expression of the high

affinity and low affinity receptor for IgE and IgE-bearing cells will allow to study if the treatment can reduce receptor expression and frequency of IgE-positive cells.

In order to determine if IgE depletion from the plasma will also reduce the allergen-specific sensitivity in allergic patients, blood samples from the patients will be exposed to serial dilutions of allergens in vitro and the upregulation of CD203c expression on basophils will be assessed by FACS analysis as described (19). Furthermore, it is planned to investigate if IgE depletion has effects on allergen-induced T cell activation and cytokine secretion. For this purpose, PBMC will be isolated from heparinised blood samples by Ficoll density gradient centrifugation and cultured with and without allergens. T cell proliferation in response to allergen will then be assessed via incorporation of 3H thymidin by liquid scintillation counting. Furthermore, the cytokine release from PBMC after allergen-stimulation will be determined in PBMC cultures using the xMAP derived Bio-Plex (BIORAD, Hercules CA, USA) fluorescent bead-based technology. Using this assay simultaneous testing of 17 human cytokines, chemokines and growth factors becomes possible in the culture supernatants. The comparison of allergen-specific T cell activation and cytokine release before and after IgE depletion will inform if IgE depletion has effects on T cell activation which may occur through IgE-facilitated allergen presentation (20).

Symptoms and Medication

During the study period patients are requested to keep a daily diary in which they will record and grade their allergy symptoms, the intake of medication and their well-being in relation to allergy. All patients are on their prescribed drug therapy, e.g. long-and short acting beta agonists**,** inhaled corticosteroids, anti-histamines, systemic corticosteroids, leukotriene antagonists. The intake of drugs will be documented in the Patient Diary during the full study period and patients will be encouraged to reduce medication intake if possible.

Respiratory function

Forced expiratory volume (FEV) is measured in the clinic using a spirometer. FEV1 is the volume of air that can forcibly be blown out in one second, after full inspiration. Average values for FEV1 in healthy people depend mainly on sex and age. Values of between 80% and 120% of the average value are considered normal.

Peak expiratory flow (PEF). Peak expiratory flow is the measurement of how much the patient can blow out of their lungs in one breath. PEF is useful for patients to perform themselves, especially when they are having a flare up of asthma. The “Vitalograph” peak flow meter from GlaxoSmith Kline is used by all patients. The results of self-measurements are documented in the Patient Diary.

Asthma Control Questionnaire

Asthma control was measured using the Asthma Control Questionnaire filled out by the patients as published by Juniper and Coworker in 1999 (21). This questionnaire includes 5 questions regarding the main asthma symptoms, one question on ß2-agonist use and another on airway calibre (forced expiratory volume 1, FEV1) (21). The English version of the questionnaire can be found in the Appendix II.

3. STUDY DESIGN

3.1. DESCRIPTION OF THE STUDY DESIGN

This clinical trial targets the clinical evaluation regarding safety and efficacy of IgE depletion to obtain CE-certification (e.g. according to MPG) for the IgE adsorber. The design is prospective, single-centric, open, controlled, randomized, comparative and interventional. After fulfilling all eligibility criteria and given Informed Consent 15 atopic asthmatic patients well characterized in their IgE reactivity profile will be randomized to either apheresis treatment with the new IgE adsorber (10 patients) or to the control group (5 patients). Subjects in the apheresis group as well as in the control group will continue their conventional pharmacological treatment during the study. Patients randomized to apheresis will be treated 3 times in a period of approximately 5-12 days with apheresis using the new IgE adsorber. During each visit 2 plasma volumes will be processed at a maximum blood flow rate of 120 ml/min and at a maximum plasma flow of 30 ml/min. Two more treatment cycles are performed after an approximately 4-5 weeks interval each. Patients randomized to the control group will serve as a reference group for the monitoring of the natural fluctuation of IgE levels and of the course of allergic asthma. Figure 1 gives an overview of the study schedule, tables 1 and 2 illustrate the schedules for the subjects in the apheresis and control group in more detail.

At the screening visit 100 ml blood will be drawn for safety and immunological analysis in both study groups. Furthermore subjects will undergo the screening procedure including clinical examination, lung function testing and titrated skin prick testing. At each of the 3 treatment visits (visits 2-4) another 30 ml of blood will be collected in the apheresis group. Subjects in the control group will have only two visits (i.e., visits 2 and 3) and two blood samples of each 20 ml will be collected for safety and immunological analysis. These cycles will be repeated twice after approximately 4-5 weeks. In between, there will be visits (Apheresis group: visits 5, 9; Control group: visits 4, 7) where physical examination, lung function testing, skin prick testing and blood analysis (safety, immunology: 80 ml) will be performed. Between weeks 13-14 and at week 16 there are two more visits during which physical examination, lung function testing, skin prick testing and blood sampling (safety, immunology) will be performed. This clinical trial

targets the clinical evaluation to obtain CE-certification (e.g. according to MPG) for the IgE adsorber.

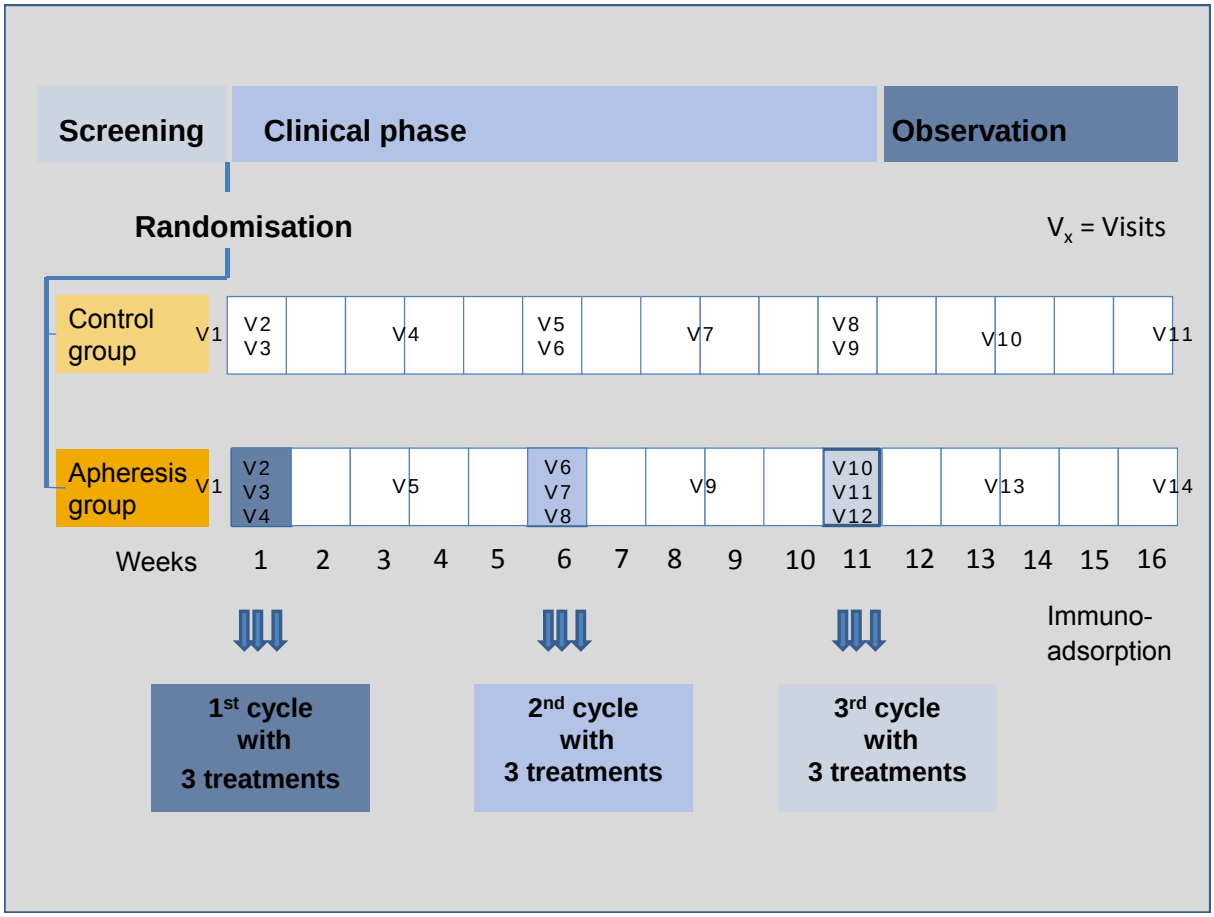


Figure 1: Overview of the study schedule

Table 1 provides an overview of all interventions and the amount of blood taken in the apheresis study group. In total an amount of 710 ml blood is taken during the time period of four months.

Table 1	**Visit**	**Examination**
Screening	V1	Blood sampling (100 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		Distribution of 1st Patient Diary to patient
Week 1 **1st apheresis cycle**	V2-V4 (at least 1 day off between visits)	Blood sampling (3x30 ml)
		ECG, blood pressure, heart frequency, body temperature, blood gas analysis
Week 2	-	

Week 3-4	V5	Blood sampling (80 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		ECG, blood pressure, body temperature
		Return of 1st and start of 2nd Patient Diary
Week 5	-	
Week 6 **2nd apheresis cycle**	V6-V8 (at least 1 day off between visits)	Blood sampling (3x30 ml), urine analysis
		ECG, blood pressure, heart frequency, body temperature, blood gas analysis
Week 7	-	
Week 8-9	V9	Blood sampling (80 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		ECG, blood pressure, body temperature
		Return of 2nd and start of 3rd Patient Diary
Week 10	-	
Week 11 **3rd apheresis cycle**	V10-V12 (at least 1 day off between visits)	Blood sampling (3x30 ml), urine analysis
		ECG, blood pressure, heart frequency, body temperature, blood gas analysis
Week 12	-	
Week 13-14	V13	Blood sampling (80 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		ECG, blood pressure, body temperature
Week 15	-	
Week 16	V14	Blood sampling (100 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		Return of 3rd Patient Diary to investigator

Table 1: Interventions and blood amount taken in the apheresis study group. The given time table may be prolonged in case all flexible times are taken.

Table 2 shows an overview of all interventions and the amount of blood taken in the control group. In total an amount of 500 ml blood is taken in the time period of four months.

Table 2	**Visit**	**Examination**
Screening	V1	Blood sampling (100 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		Distribution of 1st Patient Diary to patient
Week 1	V2,V3 (beginning and end of week)	Blood sampling (2x10 ml), urine analysis
		ECG, blood pressure, body temperature, blood gas analysis
Week 2	-	
Week 3-4	V4	Blood sampling (80 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		ECG, blood pressure, body temperature
		Return of 1st and start of 2nd Patient Diary
Week 5	-	
Week 6	V5, V6 (beginning and end of week)	Blood sampling (2x10 ml), urine analysis
		ECG, blood pressure, body temperature, blood gas analysis
Week 7	-	
Week 8-9	V7	Blood sampling (80 ml)
		Physical examination and lung function test
		Skin prick test
		ECG, blood pressure, body temperature
		Return of 2nd and start of 3rd Patient Diary
Week 10	-	
Week 11	V8 Monday V9 Friday (+3 days)	Blood sampling (2x10 ml), urine analysis
		ECG, blood pressure, body temperature
Week 12	-	
Week 13-14	V10	Blood sampling (80 ml), urine analysis
		Physical examination and lung function test

		Skin prick test
		ECG, blood pressure, body temperature, blood gas analysis
Week 15	-	
Week 16	V11	Blood sampling (100 ml), urine analysis
		Physical examination and lung function test
		Skin prick test
		Return of 3rd Patient Diary to investigator

Table 2: Interventions and amounts of blood taken in the control group. The given time table may be prolonged in case all flexible times are taken.

3.2. JUSTIFICATION FOR THE STUDY DESIGN

The primary objective of the study is to assess the ability of the IgE adsorber to deplete specifically IgE antibodies from patients suffering from allergic asthma. Since total and allergen-specific IgE levels are subject to fluctuations depending on allergen exposure (22) a control group which is not subjected to apheresis has been included in addition to the apheresis group. Both groups will continue to take their regular anti-asthma medications during the full study, so that there will be no disadvantage for the subjects when participating in the trial. The apheresis group may benefit clinically through the depletion of the pathogenic IgE antibodies which will be assessed by several investigations (skin testing, lung function testing, assessment of symptoms, well-being and medication intake as well as a battery of immunological parameters known to be associated with allergy) but this is not a primary objective of this study. Importantly, there will be a rigorous assessment of the safety of the apheresis treatment with the IgE adsorber by continuous assessment of safety laboratory parameters and physical examinations of the subjects.

3.3. ASSIGNMENT OF PATIENT NUMBER

The code for the subjects will have two digits for the study center 01 and three digits for the local subject number, e.g. 01012 for the 12th patient of the one center.

3.4. RANDOMIZATION, ALLOCATION OF PATIENTS

After screening and determination of total IgE levels patients will be ranked according to total IgE levels and then randomly assigned in a ratio 2:1 to two groups:

- Apheresis

- Control

Randomly permuted blocks of fixed size will be used for allocation of patients to treatment sequences. The study statistician will generate randomization lists based on code generated in SAS V9.2. The corresponding code will be filed inclusive the random seed which was used. If a patient is eligible to participate in the clinical investigation and has signed the Informed Consent, the patient will be randomly assigned to one of the two treatment groups using the centralized fax randomization by FME, Bad Homburg. The random allocation procedure will be carried out in the following way: the center has to send a fax to Fresenius Medical Care Deutschland GmbH (Fax Number +**49 6172 609 2386**) providing the following data for the randomization on the Patient Entry Form:

- Name, mailing address, fax and telephone number of the center,
- Patient identification number,
- Patient's year of birth.

3.5. BLINDING AND DECODING

Not applicable.

3.6. STUDY DURATION

After getting approval by the Ethics Committee and the Authorities the screening of patients will start. As soon as 15 patients are recruited they are randomized to either the apheresis treatment group or the control group. It is planned that at least two patients can be treated per week.

4. PATIENTS

4.1. INCLUSION CRITERIA

General

- 18 to 50 years old
- Male and female (contraception during study participation)
- Signed Informed Consent

Study-specific

- Clinically relevant allergy to respiratory allergens confirmed by patient's history, skin-prick testing, IgE serology
- Allergic asthma (diagnosed by lung specialist)

- IgE concentration in blood at least 300 kU/L

4.2. EXCLUSION CRITERIA:

General

- Participation in another trial within 30 days prior to enrolment
- Gravidity (pregnancy test prior to each treatment cycle)

Study-Specific

- Intake of omalizumab
- Hepatitis or HIV or malignant disease
- Specific immunotherapy in the last 6 months
- Non-allergic asthma
- Auto-immune diseases
- Hypocalcaemia
- Intake of ACE-inhibitors (discontinuation according to half-time before treatment possible)
- Uncontrolled bleeding and coagulation disorders
- Severe cardiovascular disease
- Severe cardiac arrhythmias
- Severe systemic infection
- Unability to tolerate therapeutic apheresis procedures (hypersensitivity associated with previous apheresis sessions)
- Inadequate peripheral venous access
- Condition in which acute fluid shifts may cause congestive heart failure
- Established or suspected intra-cranial disease where fluid imbalance or pressure changes could exacerbate the disease
- Impaired renal function
- Clinically significant hypotension or borderline hypotension where a further drop in blood pressure could be harmful

4.3. WITHDRAWAL CRITERIA/DROP-OUTS

Once a patient has been included into the clinical investigation (i.e. has been treated with the medical device) the investigator will make every reasonable effort to keep the patient in the clinical investigation.

However, if the investigator has to withdraw a patient from the treatment period or if the patient refuses further study participation, a complete final examination (like the visit after 16 weeks at

study end) should be performed. The results of the final examination and the reasons for withdrawal must be recorded on the Case Report Form.

Patients who are withdrawn from the clinical investigation due to incidents / serious adverse device effect will be treated and followed-up according to established medical practice to evaluate the course of the incident / serious adverse device effect and to ensure reversibility or stabilisation (see also chapter 7).

The following are justifiable reasons for the investigator to withdraw a patient from the clinical investigation:

- Intolerable incident / serious adverse device effect
- Severe violation of the study protocol
- Withdrawal of informed consent

In case a patient cannot be treated according to the schedule described above due to reversible conditions like, e.g., fever, treatment can be postponed accordingly without excluding the patient from the study.
Drop outs will be replaced by screening new study subjects with similar total IgE levels until the planned number for each group has been achieved

4.4. CONTROL GROUP

The control group provides a group of patients for the assessment of natural fluctuations of IgE levels. Most likely the IgE levels will not change in this group but it needs to be controlled. The control group will also allow to obtain data regarding eventual reductions in medication intake and symptoms when compared to the apheresis group..

5. STUDY DEVICE AND TREATMENT

5.1. CHARACTERISTICS OF THE MEDICAL DEVICE, ESSENTIAL REQUIREMENTS

The IgE adsorber conforms to the essential requirements according to Medical Device Directive (93/42/EEC and its revision 2007/47/EC) apart from the aspects covered by this clinical investigation; with regard to these aspects, every precaution has been taken to protect the health and safety of the patients. The biocompatibility of materials with direct or indirect blood contact was tested according to DIN EN ISO 10993 (Biological Evaluation of Medical Devices).

The single use IgE-adsorber system comprises of the plasma separation unit (centrifuge COM.TEC) and apheresis control unit (incorporated in the COM.TEC) as well as various disposables including the IgE adsorber, a particle filter, tubing systems, NaCl- and ACD-A-

solution, a waste bag, rinsing solutions, accessories and operating instructions. Pre-rinsing with the NaCl solution supplemented with 2800 to 3000 IU heparin is mandatory for preparation of the system before treatment of the patient. Heparin is used for systemic anticoagulation, citrate is used for anticoagulation of plasma. The disposable set including the adsorber is designed for single use only.

The IgE adsorber (figure 2) consists of a column containing Sepharose as matrix material to which a ligand (BM10) with high affinity to IgE is bound. The BM10 ligand is a recombinant single-chain variable fragment (scFv12) expressed from cDNA coding for the heavy and light chains of a murine monoclonal antibody specific to IgE.

The IgE adsorber for clinical use is sterilized by gamma-irradiation. The housing consists of a cylindrical corpus (material: polycarbonate), two sieves (material: polyester) with a mesh width of 20 µm (± 3.5 µm) and two flanges (material: polycarbonate). The connections for the in- and out-coming accesses are female luer-lock-connectors.

The middle-connector is only used for the filling of the adsorber. After filling the connector is closed with a cap and secured against opening with a safety cover.

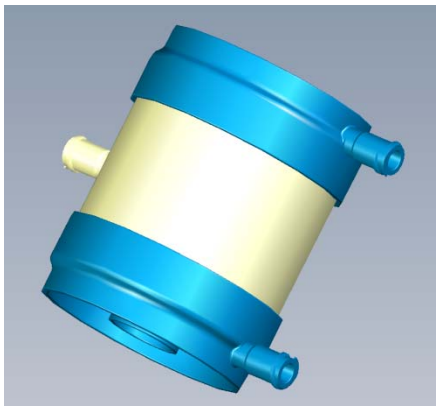


Figure 2: Investigational product: IgE adsorber without label

5.2. TREATMENT PROCEDURE

A schematic overview of the treatment procedure provides figure 3: in the first step the IgE adsorber is rinsed by gravity with 2000 ml of 0.9% NaCl to discharge the sterilization buffer and to remove residuals arising from sterilization and storage.

The patient is connected to the COM.TEC cell separator treatment set by peripheral venous access on both arms. The anticoagulation during the treatment will be maintained by continuous citrate dosage (1:14 -1:25) into the blood line. A heparin bolus is given before start of treatment. The COM.TEC cell separator separates plasma from cellular blood components by centrifugation and processes the obtained plasma through the IgE adsorber

where the adsorption process takes place. The plasma is then re-unified with the blood cells in a bubble catcher of the cell separator and returned to the patient. After treatment the residual blood in the blood lines is displaced by physiological saline and re-infused to the patient. The plasma separation device which will be used in combination with the IgE adsorber is depicted in figure 4.

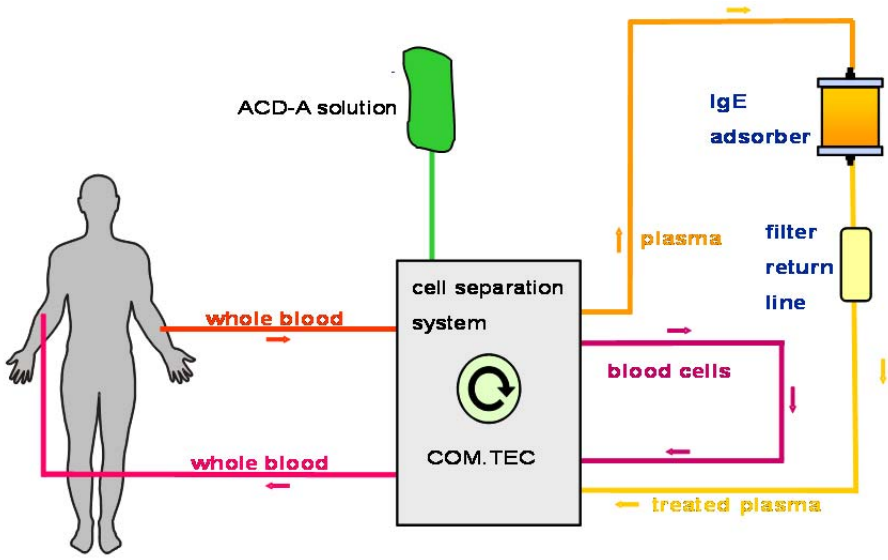


Figure 3: Schematic overview of the apheresis treatment with the IgE adsorber

Figure 4: Photo of the IgE adsorber with the plasma separation unit COM.TEC

5.3. SUPPLY, PACKAGING, LABELING AND STORAGE

FME will provide the investigator with a sufficient number of medical devices (with accessories and disposables) to perform the clinical investigation.

The label on the IgE adsorber will include the following information according to the Essential Requirements of the MDD:

- legal manufacturer (name, address)
- device identification / study number
- lot number
- storage instructions
- manufacturing date
- expiry date
- sterile state with kind of sterilization
- name of the investigator
- the term: 'Device intended for clinical investigation only'

Until applied to the patients, the medical device will be stored in a securely locked area, only accessible to authorized personnel.

At the end of the clinical investigation any unused study material will be returned to FME.

5.4. MEDICAL DEVICE ACCOUNTABILITY (DELIVERY, RECEIPT, USE, RETURN)

IgE adsorbers will be shipped to the clinic after the clinical Monitor confirmed the initiation of the centre. The site confirms receipt of the material by faxing the signed and dated delivery note to the fax number indicated on the note.

In parallel the Accountability Log describing delivery (provided in the Investigator's Site File) will be completed.

All study material will be stored at room temperature with the exception of the IgE adsorber that is stored at 2 to 8°C under controlled conditions in a refrigerator that can be locked.

Upon every patient treatment visit the Device Dispense Log (provided in the Investigator's Site File) will be completed by entering the type of device, date, batch number, patient identification, and signature.

At the end of the trial all remaining unused materials (also all destroyed unassigned material) will be listed on the Device Return Log (provided in the Investigator's Site File). All unused materials will be returned to FME. Used disposables will be disposed locally following to all applicable safety and environment protection guidelines.

The investigator is responsible for the accountability of the study materials at the investigating centre. Any use of the materials is to be documented per patient on the Case Report Form. At the end of the clinical investigation the investigator will have to explain any discrepancies between delivery, use and return of study materials (accountability).

For this clinical study the centre will be equipped with two CE-certified apheresis devices (COM.TEC) to perform the treatments. Before use all personnel is trained by the sponsor on handling of the devices and the devices have to pass technical safety controls. After completion of the study the devices will be returned to the sponsor.

5.5. CONCOMITANT MEDICATION

Subjects in the apheresis as well as in the control group will continue to take their anti-asthma medication as needed. All application of medication during the study is documented in the Case Report Form and in the Patient Diary.
Medication with impact on prolongation of bleeding time e.g. due to inhibition of platelet aggregation, such as acetyl salicyl acid should be avoided during the treatments of the clinical study and during the intervals between apheresis treatments.

6. STUDY PROCEDURES

6.1. SUMMARY OF PROCEDURES

An overview of the study course for each patient is provided in the Study Schedule of the Synopsis. The procedure is described in detail in the following paragraphs:

6.2. DETAILED DESCRIPTION OF INVESTIGATIONS

A description of the planned time-points and the chosen types of investigations is given under 6.3.2.

6.2.1 Screening period

During the screening period the investigator at the pulmonology department informs patients eligible for study participation in respect to age, asthma symptoms and their allergy profile about the clinical study and answers all questions; moreover, the patient obtains two copies of the written Patient Information sheet. During the screening the eligibility of the patient for participating in the study in respect to pulmonological parameters (classification of mild to moderate asthma) is proven by the pulmonology investigator within the clinical routine. Afterwards the patient will be checked by the Principle Investigator at the apheresis department for eligibility of apheresis therapy.

The Principle Investigator for the apheresis will again explain the objectives, risks and scope of the trial. The patient will be informed in detail by the Patient Information and a verbal explanation. The Principle Investigator for the apheresis checks inclusion and exclusion criteria for the apheresis treatment; if a criterion is not met, this patient will not be recruited for the clinical trial. If the patient fulfills the study criteria and agrees to participate in the study he/she will sign the two copies of the Patient Information sheet in addition to the Principle Investigator. One copy will be handed to the patient, the other copy stays at the study centre. The patient receives a five digit code number in the order of inclusion.
Demographic and anamnestic data of the recruited patients are documented in the CRF.

6.2.2 Treatment period

Apheresis group

The patient's total volume of plasma is calculated on the basis of sex, body weight, height and haematocrit in accordance to Sprenger et al. (23). In order to obtain an optimal reduction of IgE with respect to efficacy and patient safety, about two times the patient's plasma volume will be treated.

Prior to each immunoadsorption treatment, information on occurrence of adverse events since the previous visit will be collected. A venous blood sample will be taken for lab analysis for safety and immunology analysis at maximum 5 minutes prior to treatment start. Heart rate is taken continuously, the blood pressure will be recorded every 60 minutes during treatment.

Before each apheresis treatment, the IgE adsorber system will be prepared in accordance with the Instruction for Use.

"Pre-treatment" and "post-treatment" blood samples will be taken from the indwelling venous cannula on the inlet side (blood flow from patient to device) according to the laboratory manual.

The investigator should ensure that the 2-fold plasma volume is treated for each patient in each treatment session to create comparable conditions. The blood flow rate will be limited to a maximum of 120 ml/min in all treatments. All efforts should be made to keep the blood flow rate constant during all treatments.

The treatment visits of one treatment cycle are performed on three days in a time span of 5 to 12 days. The first treatment cycle is followed by a period of 4 to 5 weeks without apheresis. However the patients will come to the clinic once between the treatment cycles in order to give a blood sample for immunology assessment, to perform lung function tests, skin prick testing and physical examination. This cycle is repeated twice and the final examination will take place after around 16 weeks (+ 2 weeks allowed) for each patient.

Control group

At time points corresponding to the apheresis treatment, subjects from the control group will have their visits. However, there will be only two visits (i.e., visits 2 and 3) and two blood samples of each 20 ml will be collected for safety and immunological analysis. These cycles will be repeated twice after approximately 4-5 weeks. In between, the patients will come to the clinic once between the treatment cycles in order to give a blood sample for immunology assessment, to perform lung function tests, skin prick testing and physical examination. This cycle is repeated twice and the final examination will take place after around 16 weeks (+ 2 weeks allowed) for each patient. In week 13 or 14 and at week 16 there are two more visits during which physical examination, lung function testing, skin prick testing and blood sampling (safety, immunology) will be performed.

6.2.3 Follow-up

The final visit for all study participants will be 16 to 18 weeks after the first treatment.

Patients, who leave the study early, will have the final visit at their time of leaving for taking a blood sample and physical examination. Body temperature, pulse, blood pressure are taken, a urine analysis is performed and CRP, clinical chemistry, haematology, IgE concentration and fibrinogen are determined in the blood.

At the end of the clinical trial the investigator documents, whether the patient has completed the study according to study protocol, or whether protocol violations did occur.

6.3. METHODS

6.3.1 Laboratory Analysis

Blood samples will be taken from the indwelling venous cannula on the inlet side (blood flow from patient to device) or by separate venipuncture when no apheresis is done.

All blood samples will be processed, transported and analysed as described in the laboratory manual. Safety and immunological analysis will be carried out in a blinded manner under conditions of good laboratory practice in the central laboratory of the Vienna General Hospital and at the Department of Pathophysiology and Allergy Research for routine and immunological analyses, respectively.

After laboratory analysis, the laboratory sends a copy of the laboratory report (including the reference ranges) with out-of-range values highlighted to the investigator and the project manager. Upon receipt the investigator assesses the clinical significance of out-of-range lab values on the lab report and confirms review by signing and dating the document.

All clinically significant lab values will be treated and documented as Adverse Events or Serious Adverse Events, depending on seriousness judged by the investigator (see chapter 7).

6.4. DOCUMENTATION OF PATIENT DATA

6.4.1 The Case Report Form

All patient data generated during the study will be recorded on the Case Report Forms (CRFs) provided by the sponsor FME. These forms are specifically designed to meet the data recording requirements of the clinical study protocol. Only the investigator and co-workers authorized by the investigator (as listed on the form "Site Responsibility Log" provided by FME) are allowed to fill-in the CRFs or to make corrections. Each CRF will be reviewed, signed and dated by the investigator after completion. CRFs should be filled-in with a black ballpoint pen. Entries must be easily legible and complete. If data are missing this has to be explained on the CRF. For all laboratory data the units or any transformation of units must be clearly defined.

Corrections of wrong entries are done by crossing out the entry in such a way that the original data remain legible. Correction fluids are not allowed. The correct entry will be made close to the erroneous entry. The correction must then be signed (e.g. with initials) and dated. In addition, in case of essential corrections of data (e.g. primary parameter, incident) the reason for the correction must be entered.

At the end of the study the original CRFs will be returned to FME. The investigator will be provided with duplicates or photocopies of the CRFs. All records and documents pertaining to the conduct of the study (particularly CRFs, patient informed consent forms, accountability sheets, original data including the patient files) must be retained by the investigator for at least 15 years after termination of the study. Retrospective identification of all patients must be possible at any time.

6.4.2 Documentation of drop-outs

If a patient drops out from the study the reason must be documented on the Case Report Form. See also 8.2 (statistics), 4.3 (withdrawal criteria / drop-outs), and 7 (assessment of safety).

6.4.3 Patient Diary

Patients of the apheresis as well as of the control group get a Patient Diary in which they will record and grade their allergy symptoms, the intake of medication and their well-being in relation to allergy every day.

7. ASSESSMENT OF SAFETY

7.1. DEFINITIONS

The following terms and definitions apply as accordingly given in ISO 14 155 “Clinical investigation of medical devices for human subjects – Good clinical practice”. Other references are used in case of complementary information and are specified if applicable.

7.1.1 Adverse event (AE)

“Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device

NOTE 1 This definition includes events related to the investigational medical device or the comparator.

NOTE 2 This definition includes events related to the procedures involved.

NOTE 3 For users or other persons, this definition is restricted to events related to investigational medical devices.”

7.1.2 Adverse device effect (ADE)

“Adverse event related to the use of an investigational medical device.

NOTE 1 This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.

NOTE 2 This definition includes any event resulting from use error or from intentional misuse of the investigational medical device.”

7.1.3 Device deficiency

“Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance

NOTE Device deficiencies include malfunctions, use errors, and inadequate labelling.”

7.1.4 Serious adverse event (SAE)

“Adverse event that

a) led to death,
b) led to serious deterioration in the health of the subject, that either resulted in
    1) a life-threatening illness or injury, or
    2) a permanent impairment of a body structure or a body function, or
    3) in-patient or prolonged hospitalization, or

4) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,

c) led to foetal distress, foetal death or a congenital abnormality or birth defect

d) led to the occurrence of a malignant tumor (MPG Austria §3 (16))

NOTE 1 Planned hospitalization for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event.

MEDDEV 2.7/3: "Device deficiencies that might have led to a serious adverse event if:

a) suitable action had not been taken or
b) intervention had not been made or
c) if circumstances had been less fortunate

shall be reported as SAE."

7.1.5 Serious adverse device effect (SADE)

"Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event"

7.1.6 Unanticipated serious adverse device effect (USADE)

"Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

NOTE Anticipated serious adverse device effect (ASADE) is an effect which by its nature, incidence, severity or outcome has been identified in the risk analysis report."

Comment: Anticipated serious adverse device effects of the investigational device are listed in this study protocol (7.5.1).

7.1.7 Incident

"Any malfunction or deterioration in the characteristics and/or performance of a device, as well as any inadequacy in the labelling or the instructions for use which, directly or indirectly, might lead to or might have led to the death of a patient, or user or of other persons or to a serious deterioration in their state of health (MEDDEV 2.12-1)."

Comment:

- The term "serious adverse device effect" and the term "incident" share common characteristics. However, the term incident is used within the established incident reporting system concerning CE marked medical devices.
- All SADEs and all device deficiencies that might have led to an SADE of the CE marked

medical devices used in the study are subject to the incident reporting as well.

- In this study all devices except the IgE adsorber are CE marked medical devices.

7.1.8 Serious public health threat

"SAE which indicates an imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for other patients/subjects, users or other persons or a new finding to it.

This includes:

- events that are of significant and unexpected nature such that they become alarming as a potential public health hazard, e.g. human immunodeficiency virus or Creutzfeldt-Jacob Disease.
- the possibility of multiple deaths occurring at short intervals (MEDDEV 2.7/3, MEDDEV 2.12/1)."

7.1.9 Causality assessment

For each event the investigator has to give a statement whether there is a reasonable causal relationship to the investigational medical device. The expression 'reasonable causal relationship' means to convey in general that there is evidence or argument to suggest a causal relationship to the investigational medical device.

This assessment can be preferably in a simple "yes" or "no" answer. Alternatively a gradual assessment can be applied (e.g. "definitely related" and "likely" qualify as reasonable causal relationship; "unlikely" and "not related" do not). Which assessment is chosen depends on complexity and focus of the assessment.

7.1.10 Severity assessment

The severity (intensity) of an event or effect is a relative estimate, made by the investigator or qualified designate, based on the comparison with the most severe case encountered in past training and clinical experience.

Each event or effect will be classified in one of the three following categories and will represent the maximum intensity reported during the evaluation period in question.

Mild	an adverse event, usually transient, requiring no special treatment, and generally not interfering with usual everyday activities.
Moderate	an adverse event, which may be improved by simple therapeutic measures; the event is discomforting to such a degree that it interferes with usual everyday activities.

Severe	an adverse event which is incapacitating and prevents the pursuit of usual everyday activities; simple therapeutic measures cannot ameliorate the event.

Comment:

The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on patient/event outcome or action criteria.

7.1.11 Outcome assessment

The outcome of the adverse event can be as follows and must be documented:

(1) Resolved without sequelae	fully recovered.
(2) Resolved with sequelae	partially recovered (e.g. event resolved but residual permanent disturbance of health).
(3) Ongoing	
(4) Fatal	death, which is reasonably related to this specific reported event/reaction. If the death is not reasonably related to this event/reaction, the outcome reached at time of death has to be given (e.g. ongoing). Please mind that death is always the outcome of an event and will not be accepted as diagnosis. Instead the diagnosis leading to death has to be provided.
(5) Unknown	only if absolutely no information on the patients outcome can be collected, e.g. in the case the patient is lost for follow up.

7.2. DOCUMENTATION AND REPORTING BY THE INVESTIGATOR

All AEs occurring during the study must be completely documented by the investigator on the AE page of the Case Report Form.

The documentation of AEs starts after the patient signed the Informed Consent and continues until the last study visit.

Seriousness and causality shall be determined by the investigator according to the definition given above, taking into account the comments presented.

The investigator shall report in writing all Serious Adverse Events whether related or not to the investigational device no later than 24 hours after the first knowledge of the SAE to the responsible Project Manager by email or fax, in case of justified delay not later than within 3

calendar days after the occurrence of the event (MEDDEV 2.7/3). In case of urgency the investigator has to inform the sponsor in parallel by phone (preferentially Project Manager, if not available Clinical Safety Officer). In case of suspicion of serious public health hazard that becomes evident on weekends and bank holidays the investigator has to inform the Internal Security Department.

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On weekends and bank holidays: Internal Security Department
Phone: +49-6172 609 2240

The immediate report must be in writing on the provided SAE report form. It has to be in English language and include at least the following information:

1. a diagnosis and short description of the SAE,
2. an assessment if there is a reasonable causal relationship between the investigational medical device and the adverse event,
3. an identifiable subject (e.g. study subject code number, age),
4. an identifiable reporting source.

Follow-up reports shall be sent to the Project Manager until the complete information requested on the SAE form is obtained. If appropriate, further documents giving more detailed information may be added. Once sent to the sponsor, the reporting form together with the complete accompanying documentation has to be placed in the investigator's file.

The investigator shall also immediately inform the sponsor per email about adverse device effects or device deficiencies that could lead to adverse device effects (see also 7.4) and document them in the Case Report Form.

All non-serious adverse events have not to be immediately reported but must be documented by the investigator in the Case Report Form. Independent from the reporting to the sponsor, the investigator has to comply with the national or local requirements of adverse event/incident reporting by the investigator/user of the medical device to the relevant competent authorities and/or ethics committee of the study centre.

The following timelines, starting with the first knowledge by the investigator, will be followed for the reporting of AEs:

	When	To whom		
		AGES/BASG	EC	Sponsor
SAE	Within 24 hours per email using SAE Form	-	-	X
SADE	Within 24 hours per email using SAE Form	-	X	X
Incident	Within 24 hours per email using SAE Form	X	X	X
ADE	Within 24 hours per email		-	X
Non-serious AE	In CRF upon occurrence		-	X

7.2.1 Follow up of adverse events

Adverse events or adverse device effects shall be followed by the Investigator until their resolution or until the adverse event or adverse device effect is recognized as permanent or stable condition by the Investigator. Follow-up investigations may be necessary according to the Investigator's medical judgement. In this situation, the follow-up investigations do not have to be documented in the CRF but must be noted in the source documentation.

If a non-serious adverse event or adverse device effect is still ongoing at the last regular clinical investigation visit, the outcome will be documented in the CRF as "ongoing"; in the case of loss of follow-up as "unknown".

If a serious adverse event is still ongoing at the last regular clinical investigation visit, it will be followed at least until 30 days after the last clinical investigation visit. In cases of serious adverse device effects, which are considered as related to the investigational medical device the follow-up has to be performed until their resolution or until the serious adverse device effects will be recognized as a stable condition by the Investigator.

7.3. DOCUMENTATION AND REPORTING BY THE SPONSOR

The sponsor is responsible for the classification of adverse events and ongoing safety evaluation of the clinical investigation. The sponsor shall

- review the investigator's assessment of all adverse events and document in writing their seriousness and relationship to the investigational device.
- review all device deficiencies and determine and document in writing whether they could have led to a serious adverse device effect.
- communicate both opinions to concerned parties (ethics committee, regulatory authorities) in case of disagreement between sponsor and principal investigator.
- report or ensure the reporting, of all serious adverse device effects and device deficiencies that could have led to a serious adverse device effect, to the ethics committee by the principal investigator
- report to regulatory authorities, within the required time period, all serious adverse events and device deficiencies that could have led to a serious adverse device effect
- ensure that the ethics committee and the regulatory authorities are informed of significant new information about the clinical investigation.
- determine whether the risk analysis needs to be updated and assess whether corrective or preventive action is required in case of serious adverse device effects and device deficiencies.

The following timelines, starting with the first knowledge of the sponsor, will be followed for the reporting of AEs by the sponsor:

	When	**To whom**		
		AGES/BASG	**EC**	**PI**[1]
Serious public health threat	Within 2 days after awareness of public health threat	X	X	X
SAE / SADE	At the latest after 7 days after awareness of SAE per email	X	in case of corrective actions	in case of corrective actions and based on the perceived risk
Non-serious AE / ADE	Final clinical investigation report (publication)	X	X	X

SAEs will be reported using SAE reporting form to: inspektionen@ages.at

[1] Participating Investigators

7.4. SAFETY MONITORING AND EARLY STUDY TERMINATION/SUSPENSION

The patient's safety will be continuously monitored by the investigator during the clinical investigation. To decrease the risk to the study participants, sequential exposure to the new therapy is planned. After the first patient successfully completes the three apheresis treatments in the first week and there are no safety concerns the next two patients will be exposed to the study therapy in the second week. Depending on the safety assessments the next two patients will be treated and so on. It is the duty of the principal investigator to determine whether any adverse device effect (or device deficiency that could lead to adverse device effect) have occurred that could preclude the safe application of the therapy to the further study participants. The principal investigator shall immediately inform the sponsor of any adverse device effects (or device deficiencies that could lead to adverse device effects).

If considered necessary, the investigator may decide to withdraw subjects from the clinical investigation as a consequence of an adverse device effect or any other relevant medical reason. If a subject is withdrawn due to an adverse device effect a final examination should be performed and documented in the source documentation and in the CRF.

The overall patient's safety in the study will be monitored by the sponsor and the Principal Investigator. If considered necessary, the sponsor may decide to prematurely terminate the entire clinical investigation for the patients' safety or any other relevant medical reason.

7.4.1 Safety monitoring team sessions

The Principle Investigator leads the clinical study and takes care for the safety of the subjects. He will receive assistance by a safety monitoring team (SMT) established by the sponsor. Overall at least 3 SMT sessions are planned to discuss patients' safety. The first session will take place after the first patient completed the first treatment cycle. The second session will take place after at least 5 patients completed the first cycle but before the second cycle is started. The third session will take place after at least 5 patients completed the second cycle but before the third cycle is started. Additional sessions can be organized if needed.

Data to be evaluated during the SMT sessions consist of:

- SAE reports
- Available laboratory parameters of patients included in the study

The SMT sessions will be hold as telephone conferences or by other forms of documented communication tools. Each SMT session consists of at least the Principle Investigator and a representative of the sponsor, favourably the Clinical Safety Officer. If appropriate, further persons can be invited. The Project Manager is responsible for organizing the SMT sessions.

The aim of the SMT is to assess whether there is an indication of adverse device effects. Moreover SMT will recommend on further study continuation and on any modification to the study protocol needed for safety reasons e.g. stopping rules for a single patient.

7.5. GENERAL SAFETY CONSIDERATIONS

All additional risk – compared to the immune adsorbers on the market – in this study is attributable to the new ligand. The IgE adsorber bead carrier material is used for years in immunoadsorption without any safety concerns. Preclinical data did not show significant changes to the biocompatibility results obtained with the current IgE ligand (data given in the Investigator´s Brochure), therefore, we do not expect the new IgE adsorber material to be of any additional risk to the study participants. Nevertheless as this is the first in human study of the IgE adsorber the health status of the study participants will be closely monitored and the safety will be assured by sequential exposure to the new therapy, AE/SAE reporting and monitoring of safety parameters.

7.5.1 Specification of anticipated adverse events and safety parameters

As this study is the first clinical investigation with the IgE adsorber anticipated adverse events can only be concluded from similar immune adsorption columns using the same matrix material but different ligands. Such columns are the reusable double-column systems IMMUNOSORBA® (with protein A as ligand) and GLOBAFFIN® (with a synthetic ligand deriving from protein A):

Adverse events associated with the conduct of immunoadsorption

Side effects possibly occurring in conjunction with plasma separation and immunoadsorption treatment are the same as those that may also occur with other extracorporeal treatments:

- hypocalcemia
- dizziness
- chills
- headache
- fatigue
- hypotension
- paraesthesia
- oedema
- tachycardia
- arrhythmia
- haematoma (at site of puncture)

Anticoagulation is necessary for the conduct of any extracorporeal treatment, also apheresis.

In case sufficient anticoagulation cannot be achieved clotting within the extracorporeal system occurs. For immune apheresis a combination of heparin and citrate (ACD-A) anticoagulation is usually used. In general, heparin administration can be associated with increased bleeding tendency (demonstrated e.g. as enhanced occurrence of haematoma), thrombocytopenia (HIT-1 and HIT-2) and increases of serum transaminases and Gamma-Glutamyl Transferase (24). Citrate anticoagulation can result in hypocalcaemia and alkalosis. These complications are manageable by monitoring of ionized calcium and acid-base status as well as by adjustments of ACD-A and calcium dosages (25). Monitoring of ionized calcium and acid-base status is a part of the study protocol. Supplementation of intravenous calcium may be necessary.

Apheresis has also potential risk of complement activation, haemolysis, indirect impairment of liver or kidney function, or unintended adsorption of blood compounds. All the above possible adverse side-effects are monitored by the measurement of appropriate laboratory parameters (see chapter 2.3.3.).

Thus further expected ADEs are:

- Deviations in coagulation parameters due to anticoagulation
- A decrease of albumin and fibrinogen and plasma/blood components of about 15% due to dilution effects and treatment related small amounts of plasma loss

The specific desired treatment effects such as:

- • A decrease of IgE
- • A decrease in total protein due to removal of IgE

also fulfill the definition of expected ADEs. These ADEs however do not require immediate reporting of the Investigator to the sponsor.

An increase in glucose concentration in case of food intake during treatment is an expected AE but no ADE.

Contraindications

The IgE adsorber should not be used in patients with:

- hypersensitivity or allergy against any materials used in the IgE adsorber column;
- patients who are currently receiving angiotensin converting-enzyme (ACE) inhibitor medications: ACE inhibitors in combination with extracorporeal treatment (especially if negatively charged surfaces are involved) may lead to anaphylactic reactions;

- patients who cannot tolerate therapeutic apheresis procedures and have previously demonstrated hypersensitivity associated with therapeutic apheresis;
- patients for whom appropriate anticoagulation cannot be maintained;
- patients who are suffering from severe cardio-vascular disease to such an extent that extracorporeal therapy is not possible;
- patients who are suffering from systemic infection;

Warnings exist for the following patient characteristics:

- patients with inadequate peripheral venous access who require placement of a central venous catheter;
- patients where acute fluid shifts may cause congestive heart failure;
- patients with established or suspected intra-cranial disease where fluid imbalance or pressure changes could exacerbate their disease;
- patients with impaired renal function where imbalance of plasma volume could be harmful;
- patients with clinically significant hypotension or borderline hypotension where a further drop in blood pressure could be harmful;

Exclusion and inclusion criteria for the patient are defined according to this risk profile.

Risks associated with participation in the clinical investigation

During the study the patients have to be connected to the extracorporeal circuit. Venopuncture is necessary for that with the possible risk of haematoma. Furthermore, blood is drawn for analysis. The amount of about 710 ml blood in total during the study period of four months for patients in the apheresis group and about 500 ml for patients in the control group, respectively, is in the range of the amount of blood donations and should not represent a risk for the patients. Haematocrit and haemoglobin concentrations are measured during the study to monitor and control the blood loss.

7.5.2 Risk/benefit assessment

The detailed results of the risk analysis are incorporated in the Investigators Brochure. In summary the health risks for the patients are manageable. The possibility of IgE apheresis may become a therapy concept leading to long-lasting improvement of allergic symptoms after IgE removal, e.g. pulmonological parameters, which should outweigh the risks

associated with the immunoadsorption treatment in this study. In summary the benefits of the study outweigh the possible risks.

8. DATA MANAGEMENT AND STATISTICS

8.1. DATA MANAGEMENT

After finalization of the study CRF and its annotation by the clinical data manager the study database will be designed using the Clintrial clinical data management system. All received CRFs, ACQ-questionnaires and patient diaries will be electronically tracked and data is entered by trained staff using double data entry. Electronically submitted laboratory data will also be incorporated into the database. After data is entered into the database, the clinical data manager will perform electronic validation checks on the data to ensure accuracy and consistency according to the data validation plan. All data discrepancy forms generated as a result of these checks will be forwarded to the site via the monitor for resolution.

After the data discrepancy forms have been completely received and resolved, all CRFs are available and medical coding is completed, the database will be locked and released for statistical analysis.

8.1.1 Medical coding

All Adverse Events will be coded according to the version 13 of MedDRA, any medication recorded will be coded according to the version 2010 of WHO-ATC.

8.1.2 Data review meeting

After data validation is finished, the clinical data manager in cooperation with the trial statistician will prepare a list of protocol violations and unresolved discrepancies. Any consequences will be discussed during a meeting of the Data Review Committee which includes clinical data manager, statistician and project manager. In particular patients will be individually assigned to different analysis populations. Additionally, exclusion of single treatments from statistical analysis is discussed, justified and documented during the meeting.

8.2. STATISTICS

8.2.1 Primary analysis

For primary statistical analysis the following hypothesis

H_0: IgE reduction after study period (3 cycles with 3 treatments) is < 50 %

will be tested against the alternative:

H_1: IgE reduction after study period (3 cycles with 3 treatments) is ≥ 50 %

Statistical analysis is based on IgE assessments before treatment 1 of the first cycle (IgE_{pre}) and after treatment 3 of the last cycle (IgE_{post}).

IgE reduction rate (%) is calculated as

$$RR = \frac{IgE_{pre} - IgE_{post}}{IgE_{pre}} \times 100$$

A one-sided one-sample t-test will be calculated on all IgE reduction rates in the apheresis group and H_0 will be rejected if $p<0.025$. Since primary analysis is based on a single statistical test no adjustment for multiplicity is considered necessary.

8.2.2 Secondary analyses

For secondary analysis of this study, reduction rates will be calculated as denoted above

- within each treatment
- within each cycle (start of first treatment to end of third treatment)

Confidence intervals will be calculated for each of the RRs, incorporating the repeated measures design where applicable.

For the control group RRs will be calculated by taking IgE assessments at comparable assessment times. Comparisons between apheresis and control group will be calculated employing linear mixed models.

Additionally, changes in pre-cycle assessments of IgE will be examined and compared.

The reduction of IgE over treatment time, as assessed in the first treatment of each apheresis patient, will be performed descriptively and graphically.

For clinical outcome data, including prick test, lung function test, microarray test, CD203c expression on PBMCs, FcEpsilonRI expression on PBMCs, IgE-positive cells on PBMCs and other immunological parameters (e.g. allergen-induced T cell activation), descriptive summary tables at study start, study end and for differences end – start will be presented by treatment group. Statistical comparisons will be performed acc. to the measurement level of each parameter (t-test, Wilcoxon test, Fisher's Exact test).

For Asthma Control data, overall scores at study start and study end will be calculated as mean of the seven-item questionnaire (21). Statistical analysis will be performed on inter-group differences as well as intra-group changes of the overall score.

Patient Diary data will be analysed accordingly where applicable.

All p-values calculated in the context of secondary analyses will be considered as purely descriptive and exploratory.

Further details regarding statistical analysis will be described in the statistical analysis plan (SAP).

8.2.3 Safety analyses

All (Serious) Adverse Events documented during the study period will be coded and presented in a patient-by-patient listing. If necessary, listing will be given by System Organ Class.

(Changes in) concomitant medication will be displayed in a patient-by-patient listing.

For all safety laboratory assessments, including hematology, clinical chemistry, electrolyte balance, thrombogenicity, complement activation and biocompatibility, descriptive statistics will be presented by treatment group (apheresis/control). Differences to baseline will be calculated between study start and end and also within each cycle, where applicable.

Vital signs (blood pressure, heart rate) and temperature will be summarized by treatment group and assessment.

ECG results (normal/abnormal) will be presented in frequency tables by treatment group and assessment.

8.2.4 Further statistical considerations

If any IgE value falls below the detection limit, the corresponding value will be set to the limit (worst case analysis). If for ethical reasons, a third treatment within a cycle is not acceptable (e.g. patient is already below detection limit after second treatment), the result of the second treatment will be carried forward to treatment three.

8.2.5 Analysis sets

For this study, the following analysis populations will be defined:

- Per-protocol-population (PP): includes all subjects who entered the study in accordance with both the inclusion / exclusion criteria without major protocol deviations and who finished the study in accordance with the study protocol.
- Intention-to-treat population (ITT=FAS): includes all subjects who were randomized and for whom complete efficacy data is available for at least one apheresis treatment (apheresis group) or at least one post baseline blood sample (control group)
- Safety-population (SP): includes all subjects who were randomized.

Assignment of patients to analysis populations will be done during the data review meeting. Primary analysis will be based on the ITT population; an additional analysis on PP will be performed to assess validity of results.
Safety analyses will be performed on the Safety Population.

8.3. SAMPLE SIZE JUSTIFICATION

Sample size calculation is based on one-sided t-test at a one-sided significance level of a=0.025. Assuming a mean IgE reduction rate of 70 % and a standard deviation of 17 % (which is based on unpublished internal data), a sample size of n=8 will provide 80 % statistical power to prove that the margin of 50 % is significantly surpassed.

To account for an expected drop-out rate of up to 20 %, a total of 10 patients will be included in the apheresis group, resulting in a final study population of n=15, including 5 control patients.

8.4. INTERIM ANALYSIS

No interim analysis is planned for this study.

9. MONITORING

9.1. FUNCTION OF THE MONITOR

The investigator will permit the FME representative (or a person authorized by FME) to monitor the study. The purposes of this monitoring are:

- To assess the progress of the study,
- To review the compliance with the clinical study protocol,
- To discuss any problems,
- To check the CRFs for accuracy and completeness,
- To verify CRF's content against source documents. This may also include past patient records (medical history) as far as relevant for the study.
- To check if the staff has changed and if so whether the new employees have been accurately informed about their tasks,
- To assess the status of storage, application and return of the medical device,
- To review the documentation retention,
- To check the general compliance of the study with ISO 14155 and national legislation.

The investigator and his staff will be expected to cooperate with the monitor, to be available during at least a portion of the monitoring visit to answer questions and to provide any missing information.

9.2. FREQUENCY OF MONITORING

At the study site the first monitoring visit will be performed not later than 2 weeks after the first patient has been included into the study. The second visit will be performed another 2 weeks later. All subsequent visits will be performed in weekly intervals or after the last patient at the study site has completed the study. Depending on enrolment and compliance more frequent visits will be performed.

During every visit 100% source data verification will be performed for the first patient included as well as for the Informed Consent, IgE concentration measurements, AEs and SAEs. All other parameters will be checked in 50% of patients.

9.3. RETURN OF STUDY MATERIALS

The study documentation (e.g. Case Report Forms, medical device accountability sheets, serious adverse event reports) and any unused investigational medical device will be returned to FME at the end of the study.

All machines provided by the sponsor or any third party in order to perform the study will be returned to the provider as agreed separately.

10. AUDITING

Auditing is not planned but may be performed to ensure compliance of the study with requirements of Good Clinical Practice (in ISO 14 155). The auditor works on behalf of FME, but is independent from the persons participating in the clinical study project. Audits can also be performed by health authorities.

10.1. AUDITING PROCEDURES

If an audit is performed at the study site the auditor will check the following:

- Preconditions to start the study.
- Compliance of study with study protocol.
- Source data check.
- Completeness and correctness of the patient informed consents.
- Medical device accountability.
- General compliance of study with GCP (in ISO 14 155) and national law.

10.2. AUDITING FREQUENCY

There is no audit planned so far.

10.3. AUDITING DOCUMENTATION

In case an audit had been performed the auditor will provide the investigator with an audit report after each auditing visit. This is to be kept in the investigator's trial file and must be available for inspections by supervising authorities.

11. MODIFICATIONS DURING THE STUDY

11.1. PROTOCOL AMENDMENTS

If both FME and the investigator agree upon a change or addition in the study protocol during the course of the study this has to be documented in a written protocol amendment. The amendment will be signed by the same persons who signed the study protocol. The amendment becomes part of the study protocol.

Each substantial amendment must be sent to the ethics committee and the relevant authorities (with exception of administrative amendments, i.e. written to correct printing errors).

11.2. PROTOCOL DEVIATIONS

No deviation from the study protocol is allowed unless a formal amendment is made and approved by the relevant ethics committee and authority. Deviations may only be tolerated to eliminate immediate hazard to the patients. If a protocol deviation has occurred, the investigator should document it and inform FME.

11.3. PREMATURE TERMINATION OF THE STUDY

FME has the right to terminate the study at any time. Reasons which may require termination are:

- Patient enrolment is too slow.
- The investigator fails to comply with the study protocol or legal requirements.
- Data recording is not accurate, e.g. Case Report Forms are not completely filled-in or entries are not legible.
- The incidence and/or severity of adverse device effects / serious adverse device effects in this or in parallel studies indicate a potential health hazard caused by treatment with the investigational medical device / control device.
- FME is requested by health authorities to terminate the study.
- FME decides to terminate the study due to internal reasons.

Should a termination of study occur prior to the scheduled completion, the investigator will be notified of such an action and receives instructions, if necessary, as to what final examinations are required.

12. FINAL REPORT

FME is responsible for compilation of the final report which will cover both biometrical and medical aspects of the study. The final report will be submitted to the (please select: study coordinator / principal investigator) for approval and signature. A copy of the final report will be sent to each investigator for his file.

13. ADMINISTRATIVE REQUIREMENTS

The trial will be carried out in accordance with the national requirements (e.g. the German Medical Device Act), with the international standard ISO 14155 Clinical investigation of medical devices for human subjects - Good clinical practice, and with the declaration of Helsinki, revised version.

13.1. ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD

The study protocol and relevant study-related documents will be submitted to the Ethics Committee of the Medical University of Vienna for review. Approval by the ethics committee is a pre-requisite for initiation of the clinical investigation. During the clinical investigation the ethics committee will be informed of any amendment to the study protocol and any incident / serious adverse device effect / serious adverse event (adjusted according to the procedures predefined by the ethics committee).

13.2. SUBMISSION/NOTIFICATION TO HEALTH AUTHORITY

FME is responsible for the submission of the study protocol and study-related documents to the “Bundesamt für die Sicherheit im Gesundheitswesen, BASG”.

13.3. PATIENT INFORMATION AND INFORMED CONSENT

It is the responsibility of the investigator, to give each patient prior to inclusion in the clinical investigation, full and adequate verbal and written information regarding the objective and procedures of the clinical investigation and the potential benefits, discomforts and risks involved. The investigator has to disclose the patients of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the patient. The patients must be informed that the participation in the clinical investigation is strictly voluntary, and about their right to withdraw from the clinical investigation at any time without specification of reasons in writing. The patients have to be informed that refusal to participate and a discontinuation of participation at any time will involve no penalty of loss of benefits to which the patient is otherwise entitled. The patients have to be informed that they are covered by insurance and, therefore, need the consent of the investigator if other medical treatment (e.g. to treat an intercurrent illness) is required, except in an emergency. A copy of the patient information sheet

and informed consent form (see appendix) has to be given to the patient before enrolment. The written patient information must not be changed without prior discussion with FME. Furthermore, it is the responsibility of the investigator to obtain signed informed consent from all patients prior to inclusion in the clinical investigation.

The signed informed consent forms should be filed by the investigator for possible review by FME monitors or for possible future audits where this is permitted and/or required. The investigator will confirm the receipt of informed consent from each patient by signing the appropriate page of the Case Report Form.

13.4. PATIENT INSURANCE

In accordance with the Medical Device Act FME has taken out a personal liability insurance (. Haftpflichtverband der Deutschen Industrie, Geschäftsstelle Edelsinnstraße 7-11, 1120 Wien, Austria; insurance number 1633257) to the amount of Euro 500,000 for each patient participating in the trial.

13.5. PATIENT PRIVACY

FME confirms and upholds the principle of the patient's right to protection against invasion of privacy. Throughout the study, all data which will be passed to FME will only be identified by identification numbers and date of birth. The data will be blinded correspondingly in all data analyses.

Source data verification will be done in detail by the on-site monitor at each visit or during a study audit. The source data verification will be performed without violation of national laws concerning data protection.

The patients will be informed in writing about the use of their data and have to document by their signature that they have been informed and that they consent to this.

13.6. INITIATION OF THE STUDY

The investigator may not enroll any patients prior to completion of a formal study initiation meeting with the FME monitor or the Clinical Research Manager.

The following documents will be provided in original by the investigator to the sponsor prior study start:

- Signed contracts
- Signed and dated protocol agreement.
- Current dated and signed curriculum vitae of investigator and that of his co-investigator(s).

- List of normal values of laboratory incl. methods and certificates (from the head of the laboratory in case of parameters, which are not determined at the study site).

14. AGREEMENTS

14.1. SECRECY AGREEMENT

This study protocol is provided to you as a principal investigator, potential investigator, or consultant for review by you, your staff, and the institutional review board. The information contained in this protocol is confidential and, except for the extent necessary to obtain informed consent, may not be disclosed unless such disclosure is required by national regulations or legislation. Persons to whom the information is disclosed must be informed that the information is confidential and may not be further disclosed by them.

Similarly, the investigator and his staff will not pass any other study-related information, documents or medication to third parties. All study materials and documents provided by FME, including this protocol, are property of the company.

14.2. PUBLICATION OF RESULTS

The study will be registered in EUDRACT and the NIH database for clinical studies before start. The data generated in this study, as well as the results of the study, are the property of the sponsor. No publication, disclosure or other use of the results of this study are allowed without prior written permission of the sponsor. Any publication will bear the name of employees of Fresenius Medical Care Deutschland GmbH who contributed to the study as co-authors. Publications will be written according to the principle of good scientific practice of the Medical University of Vienna. Any publication is based on the statistical final report approved by Fresenius Medical Care Deutschland GmbH. The investigator will receive a copy of the Final Report. Thereafter the manuscript for joint publication can be drafted. A joint publication of the results should be made in mutual agreement. All editorial decisions are taken jointly by the investigators and Fresenius Medical Care Deutschland GmbH. Fresenius Medical Care Deutschland GmbH is entitled to check a draft for publication latest 30 days before submission, and to advise within 30 days any necessary, reasonable supplements.

Fresenius Medical Care Deutschland GmbH is entitled to utilise all relevant data of the study for registration purposes, world-wide scientific product documentation and for publications.

14.3. PAYMENT

Financial obligations of FME for the performance of the study are outlined in a separate agreement between the investigator/institution and FME.

15. LITERATURE

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16. APPENDICES

APPENDIX I: Revised declaration of Helsinki (World Medical Association)

APPENDIX II: Asthma Control Questionnaire

APPENDIX I. REVISED DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

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9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy

volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

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C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

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APPENDIX II. ASTHMA CONTROL QUESTIONNAIRE

A German version of the questionnaire will be given to the patients in the study

ASTHMA CONTROL QUESTIONNAIRE©

Please answer questions 1–6.

Circle the number of the response that best describes how you have been during the past week

1. On average, during the past week, how often were you **woken by your asthma** during the night?	0	Never
	1	Hardly ever
	2	A few minutes
	3	Several times
	4	Many times
	5	A great many times
	6	Unable to sleep because of asthma
2. On average, during the past week, how **bad were your asthma symptoms when you woke** up in the morning?	0	No symptoms
	1	Very mild symptoms
	2	Mild symptoms
	3	Moderate symptoms
	4	Quite severe symptoms
	5	Severe symptoms
	6	Very severe symptoms
3. In general, during the past week, how **limited were you in your activities** because of your asthma?	0	Not limited at all
	1	Very slightly limited
	2	Slightly limited
	3	Moderately limited
	4	Very limited
	5	Extremely limited
	6	Totally limited
4. In general, during the past week, how much **shortness of breath** did you experience because of you asthma?	0	None
	1	A very little
	2	A little
	3	A moderate amount
	4	Quite a lot
	5	A great deal
	6	A very great deal
5. In general, during the past week, how much of the time did you **wheeze**?	0	Not at all
	1	Hardly any of the time
	2	A little of the time
	3	A moderate amount of the time
	4	A lot of the time
	5	Most of the time
	6	All the time
6. On average, during the past week, how many **puffs of short-acting bronchodilator** (eg. Ventolin) have you used each day?	0	None
	1	1–2 puffs most days
	2	3–4 puffs most days
	3	5–8 puffs most days
	4	9–12 puffs most days
	5	13–16 puffs most days
	6	More than 16 puffs most days

To be completed by a member of the clinic staff

7. FEV1 pre-bronchodilator:	0	>95% predicted
	1	95–90%
FEV1 predicted	2	89–80%
	3	79–70%
FEV1 % predicted	4	69–60%
(Record actual values on the dotted lines and score the FEV1 % predicted in the next column)	5	59–50%
	6	<50% predicted