STUDY PROTOCOL

SAFETY AND DOSE FINDING STUDY OF BM32 IN SUBJECTS SUFFERING FROM GRASS POLLEN ALLERGY

Protocol No.: CS-BM32-002

EudraCT Number: 2011-003368-64

Version 01
05. September 2011

Doc.No.: CSP-BM32-004

SPONSOR TRIAL NO.:	**CS-BM32-002**
CENTER TRIAL NO.:	3007
INVESTIGATIONAL PRODUCT:	BM32
STUDY PHASE:	II

Short title: **SAFETY AND DOSE FINDING STUDY OF BM32 IN SUBJECTS SUFFERING FROM GRASS POLLEN ALLERGY.**

Long title: Safety and dose finding study based on the effects of three subcutaneous injections of BM32, a recombinant hypoallergenic grass pollen vaccine, on responses to allergen challenge by skin testing and in the Vienna Challenge Chamber (VCC) as well as immunological responses in subjects known to suffer from grass pollen-induced allergic rhinitis. A prospective, randomised, double- blind, placebo-controlled, parallel group evaluation.

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SIGNATURE PAGE (Protocol Release)

This study is intended to be conducted in compliance with the protocol,

Good Clinical Practice and the applicable regulatory requirements.

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For the trial center:

I, the undersigned Investigator, understand that all information supplied to me by the Sponsor, or its contracted representatives, in connection with this trial and not previously published is considered as confidential information. This information includes the Investigator Brochure, Clinical Protocol, Case Report Form, assay methods, technical methodologies and basic scientific data. The data generated in this trial are the property of the Sponsor, and publication of such data can only be made by mutual agreement. Furthermore, I understand that any changes in the protocol must be approved in writing by the Sponsor.

By my signature below, I hereby attest that I have read, discussed and understood the background information concerning the investigational product. I have read and discussed this Protocol (CSP-BM32-002) and agree to carry out the trial as set out therein. I agree that the trial shall be carried out according to ICH Good Clinical Practice (GCP) standards, and accept my obligations relating to the principles that have their origin in the Declaration of Helsinki and specifically to Independent Ethics Committee (IEC), Informed Consent, and also my obligations to the Sponsor, or contracted representatives, as far as providing data, allowing monitoring and audit and quality control visits are concerned.

Obligations of confidentiality are accepted by both parties.

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Abbreviations

AE	Adverse Event
API	Active Pharmaceutical Ingredient
Biomay AG	Sponsor
BP	Blood pressure
CRF	Case report form
DNE	Digital nasal endoscopy
ECG	Electrocardiogram
EISR	Expedited Investigator Safety Report
ELISA	Enzyme linked Immunosorbent Assay
FA set	Full Analysis Set
FEV	Forced Expiratory Volume
FVC	Forced vital capacity
GCP	Good Clinical Practice
HR	Heart rate
IB	Investigator Brochure
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISAC	Immuno Solid-phase Allergen Chip
ITT	Intention to treat
MCA	Mean cross sectional area
MED	Minimum effective dose
NCS	Not clinical significant
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OSS	Other Symptom Score
PAR	Perennial allergic rhinitis
PP	Per protocol population
RAST	Radio-Allergen-Sorbent-Test
SAE	Serious Adverse Event
SAR	Seasonal allergic rhinitis
s.c.	subcutaneous
SPT	Skin prick test
TNSS	Total nasal Symptom Score
TNNSS	Total Non-Nasal Symptom Score
TOSS	Total ocular Symptom Score
VCC	Vienna Challenge Chamber

Protocol Summary

Protocol No.:	CS-BM32-002
Study Title:	Safety and dose finding study based on the effects of three subcutaneous injections of BM32, a recombinant hypoallergenic grass pollen vaccine, on responses to allergen challenge by skin testing and in the Vienna Challenge Chamber (VCC) as well as immunological responses in subjects known to suffer from grass pollen-induced allergic rhinitis. A prospective, randomised, double- blind, placebo-controlled, parallel group evaluation.
Short Title	Safety and dose finding study of BM32 in subjects suffering from grass pollen allergy
Study Phase:	II
Indication:	Allergic Rhinoconjunctivitis and/or mild asthma range 1 and 2 GINA

Rationale

Specific immunotherapy (SIT) is the only allergen-specific and disease-modifying therapeutic modality for IgE-mediated allergies. However, the current clinical use of extracts from allergy causing agents (pollen, mites, animal hair) is limited by many problems caused by the poor quality of natural allergen extracts, including poor characterization, lack of important allergens and contaminations of the API, inconvenient dosing schemes and occurrence of side effects, in the worst case severe and life-threatening anaphylactic side effects. Due to these problems the use of SIT is limited although it represents a causative and disease-modifying treatment unlike symptomatic pharmacotherapy which only mitigates symptoms. It is therefore highly desirable to develop products for SIT, which overcome these problems and provide a safe and convenient treatment so that a larger number of patients can benefit from this treatment. This promise is approaching reality with improved understanding of the molecular nature of the disease-causing allergens and the disease process itself. Pure allergens can now be obtained by recombinant expression and molecular modifications of the allergens allowing the development of vaccines with reduced side effects and greater efficacy.

The recombinant proteins constituting the grass pollen vaccine BM32 are the first members of a new generation of allergy vaccines, which have been designed to minimize side effects and to establish a convenient immunotherapy schedule for all major seasonal and perennial allergies. They have been engineered to reduce IgE reactivity and T cell reactivity but to retain the ability to induce protective IgG antibody responses against the natural allergens upon vaccination. Their safety has been studied in allergic patients by skin testing showing almost complete lack of allergenic activity.
The study is designed to investigate safety and dose-dependent effects of three subcutaneous injections of BM32 on immunological and clinical responses to grass pollen allergens in patients suffering from grass pollen-induced rhinoconjunctivitis.

Antibody responses to grass pollen allergens will be studied in patients treated with different doses of BM32 or placebo. Clinical effects will be studied by titrated skin prick testing and respiratory exposure to grass pollen allergens. The traditional single nasal allergen challenge has several problems; a single high dose intranasal allergen challenge does not replicate allergen exposure in the environment, it is unpleasant for the subject, it is difficult to compare different trials to each other as allergen doses have to be individually titrated during each study and challenges are time and staff intensive. Therefore it has nearly no value for evaluating anti-allergic treatment in patients.

The Vienna Challenge Chamber (VCC) allows a more physiological challenge than the intranasal challenge and comes close to the Park Study without the disadvantages of a Park Study. The VCC offers the opportunity to obtain assessments of response from up to 20 subjects in a several hours period even out of season and, because of the high reproducibility of this model, low variability of results is to be expected. Therefore only a rather small number of patients should be necessary to get significant results when testing active compounds. Within the Vienna Challenge Chamber subjects are exposed to sustained low-dose

allergen at a pre-determined concentration over several hours. This results in a sustained response, similar to that seen upon natural allergen exposure, which rises to a plateau after 90 to 120 min. Subjects are selected on the basis that they display a defined moderate response to the pre-determined concentration used.

Aim of this study is to determine a safe dose of BM32 which affects grass pollen-specific allergic reactions in the skin and upon exposure in the Vienna Challenge Chamber (VCC) and induces immunological changes known to be associated with clinically effective SIT.

Objectives

Primary efficacy objective:

- To assess the minimum effective dose after three subcutaneous injections of different dose levels of BM32 as compared to placebo. The effects of different dose levels of BM32 compared to placebo are evaluated by the grass pollen-specific Total Nasal Symptom Score (TNSS – nasal obstruction, rhinorrhoea, itchy nose and sneezing) of grass pollen-induced allergic rhinitis provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection (Visit 8) of the treatment.

Secondary efficacy objectives:

- To evaluate the effects of different dose levels of BM32 compared to placebo by studying the grass pollen-specific Total Non Nasal Symptom Score (TNNSS), i.e. the Total Ocular Symptom Score (TOSS) (watery eyes, itchy eyes, red eyes) and the Other Symptom Score (OSS) (cough, itchy throat, itchy ears) of grass pollen-induced allergic rhinitis provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection (Visit 8) of the treatment.

- To assess the effects of different dose levels of BM32 compared to placebo, by evaluating the Global Symptom Score (Total Nasal Symptom Score (TNSS) and Total Non-Nasal Symptom Score (TNNSS) combined) of grass pollen-induced allergic rhinitis provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection (Visit 8) of the treatment.

- To determine the effects of vaccination with BM32 versus placebo on allergen-specific skin responses by titrated skin prick testing (SPT) via measuring wheal areas at screening and at visit 7 and to evaluate the change in the threshold concentration of grass pollen extract necessary to provoke a positive skin reaction (SPT).

- To evaluate the effects of different dose levels of BM32 compared to placebo by the mean cross-sectional area (MCA) using anterior rhinomanometry (NAR) provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection of the treatment (Visit 8).

- To evaluate the effects of different dose levels of BM32 compared to placebo by FEV1 (Forced Expiratory Volume in 1 second) and FEV1/FVC (Tiffeneau-Value) as measured during challenge sessions at visit 3 and at visit 8 .

Safety and immunogenicity objectives:

Primary safety objective:

- To evaluate the relative safety and tolerability of three different dose levels of BM32 compared to placebo.

Secondary safety and immunogenicity objective:

- To determine the effects of vaccination with BM32 versus placebo on allergen-specific antibody responses.

- To determine the effects of vaccination with BM32 versus placebo on allergy related immunological parameters. In particular, antibody subtypes, T-cell responses and cytokine responses to recombinant allergens, grass pollen extract, BM32 components, and the carrier (PreS), as well as the sensitivity to recombinant grass pollen allergens and timothy grass pollen extract in allergen-induced basophil activation and in the CD203c assay will be studied.

Criteria for evaluation

Efficacy:

Primary efficacy endpoint:

- Difference in the total Nasal Symptom Score (TNSS) (nasal obstruction, rhinorrhoea, itchy nose and sneezing) between spending 6h in the VCC at screening and spending 6h in the VCC 4 weeks after the last injection of the treatment (Visit 8).

Secondary efficacy endpoints:

- Difference in the Total Non-Nasal Symptom Score TNNSS (TOSS and OSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the Global Symptom Score (TNSS and TNNSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the nasal airflow resistance (NAR) (measured using active anterior rhinomanometry) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Change in skin reaction to grass pollen allergens (SPT) before (screening) and after the treatment (visit 7) by dose titration of the grass pollen extract measuring the difference in the sum of wheal areas between these two visits and evaluating the change in the threshold concentration of grass pollen extract necessary to provoke a positive skin reaction (SPT)
- Difference in FEV1 and FEV1/FVC between Screening (visit 3) and Visit 8

Safety / Tolerability and Immunogenicity:

Safety and Tolerability Endpoints

1) Adverse events (AEs)

- Frequency of AEs concerning occurrence, seriousness, intensity and drug-relationship.
- Frequency of local reactions (injection site reactions) and systemic reactions classified by a grading scheme according to the Position Paper of the German Society for Allergology and Clinical Immunology[1]

[1] Kleine-Tebbe, J. , Fuchs, T. , Klimek, L., et al.: Die spezifische Immuntherapie (Hyposensibilisierung) mit Allergenen. Positionspapier der deutschen Gesellschaft für Allergologie und klinische Immunologie inhaltlich abgestimmt mit dem Ärzteverband Deutsche Allergologen. Pneumologie 2001; 55, pp. 438-44.

2) Rescue medication

Administration of rescue medication via total amount of rescue medication needed.

3) Vital signs and physical examination

- Blood pressure, pulse rate, body temperature, and breathing frequency during the whole study period, ECG and physical findings at screening, medication phase, evaluation phase and at the follow-up examination.

4) Laboratory

- Haematology, biochemistry, and urinalysis at screening, medication phase and at the follow-up examination.
- In women of childbearing potential, a pregnancy test will be performed at screening, medication phase, evaluation phase and at follow-up.

Immunogenicity Endpoints

- Change in allergen specific total IgG levels after 3 s.c. injection with BM32, as measured at screening and visit 7
- Change in allergen specific IgE levels after 3 s.c. injection with BM32, as measured at screening and visit 7
- Change in allergy related immunological parameters (T-cell responses and cytokine responses to recombinant allergens, grass pollen extract, BM32 components, and the carrier (PreS), as well as the sensitivity to recombinant grass pollen allergens and timothy grass pollen extract in allergen-induced basophile activation and in the CD_{203c} assay) levels after 3 s.c. injection with BM32, as measured in blood or serum samples collected at screening (Baseline) and after the last injection of the treatment (visit 7).

Statistical methods

All data obtained in this study will be tabulated with descriptive group statistics (mean, standard deviation, minimum, maximum, number of valid cases) or frequency tabulations depending on whether the variable is continuous or not (binary/ordinal).

Comparisons between treatment groups having continuous data will be performed by t-tests in case of normality or non-parametric methods (Wilcoxon-two-sample test, explorative significance level 5%), or in case of binary/ ordinal variables with Chi-square-tests (explorative significance level 5%). Comparisons within one treatment group to evaluate effects before vaccination and after will be done by paired t-tests or the Wilcoxon-signed rank test depending on the underlying distribution and scale.

Efficacy

The main efficacy parameter will be analysed by a stepwise one-sided multiple t-testing of the superiority in the difference TNSS Score of the verum groups over placebo. While determining the significance of the three respective tests the minimum effective dose (MED) can be determined by taking the lowest dose at which the tests have been shown to be significant (following Dunnett, Hochberg, Tamhane (1996)[2]).

[2] Tamhane, A.C., Hochberg, Y., Dunnett, C.W. (1996): Multiple test procedures for dose finding. Biometrics 52, 21-37.

The secondary efficacy endpoints

- Difference in the total Non-Nasal Symptom Score TNNSS (TOSS and OSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the Global Symptom Score (TNSS and TNNSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the nasal airflow resistance (NAR) (measured using active anterior rhinomanometry) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Change in skin reaction to grass pollen allergens (SPT) before (screening) and after the treatment (visit 7) by dose titration of the grass pollen extract measuring the difference in the sum of wheal areas between these two visits and the change in the threshold concentration of grass pollen extract necessary to provoke a positive skin reaction (SPT)
- Difference in the FEV1 and FEV1/FVC between Screening and Visit 8

These parameters will be analysed with t-tests in case of normality and continuous variables. In case of non-normality non-parametric methods (Wilcoxon two sample test, significance level 5%) will be applied to evaluate differences between treatment groups. For evaluation of within group differences (pre / post) paired t-Tests or Wilcoxon signed rank tests will be applied depending on the distribution and scale. A pre-post testing between visits will also be done for the main efficacy parameter.

Safety

Adverse events will be tabulated by system organ class and preferred term after medical coding using the Medical Dictionary for Regulatory Activities (MedDRA).

Special focus will be laid on local and systemic reactions counting the frequency of their occurrence using the grading scheme according to the Position Paper of the German Society for Allergology and Clinical Immunology[3].

Administration of rescue medication will be analysed by calculating the total amount of rescue medication applied and comparing the total amount of rescue medication between treatment groups via non-parametric test methods.

Vital signs, ECG and physical examination will be analysed via descriptive methods and by analysing shifts between screening and visit 7.

Laboratory data will be presented via descriptive and frequency tables, showing normal, non-normal and clinically relevant data.

Immunogenicity

Immunogenicity data as recorded by

- Allergen-specific total IgG levels after 3 s.c. injection with BM32, as measured at screening and visit 7
- Allergen-specific IgE levels after 3 s.c. injection with BM32, as measured at screening and visit 7

will be analysed via graphical profiles showing the time-dependent development of the antibodies. Comparisons between treatment groups will be done via non-parametric methods (Wilcoxon-two-sample test) by using the change in immunogenicity data; or the Wilcoxon-signed rank test comparing immunogenicity data within treatment groups.

Immunogenicity data will also deliver information concerning the optimal dose.

[3] Kleine-Tebbe, J. , Fuchs, T. , Klimek, L., et al.: Die spezifische Immuntherapie (Hyposensibilisierung) mit Allergenen. Positionspapier der deutschen Gesellschaft für Allergologie und klinicshe Immunologie inhaltlich abgestimmt mit dem Ärzteverband Deutsche Allergologen. Pneumologie 2001; 55, pp. 438-44.

Study Design

This will be a prospective, randomized, double-blind, placebo-controlled, parallel group, safety and dose-finding (concerning minimum effective dose - MED) study to evaluate the safety and tolerability of BM32 grass pollen vaccine compared to placebo and to study its effects on clinical responses to grass pollen allergen challenge and immunological responses to vaccination.

There will be 4 study groups: 3 dose levels of BM32, and placebo.

Study Population

The target number of subjects for this study is 60. To allow for drop outs this study will include up to 72 male and female subjects, aged 18 to 60 years with a clinical history of SAR during each of the last two grass allergy seasons, and a positive skin prick test to grass allergen within 12 months prior to Visit 1.

Study Assessments and Procedures

Subjects will undergo screening 3 - 28 days prior to study start. During screening they will undergo a qualifying challenge session (6 hours) to check that they have a clinically relevant response to allergen in the chamber as well as for stratification into the four study groups.
A clinically relevant response in the chamber is defined as a TNSS (total nasal symptom Score = nasal obstruction, rhinorrhoea, itchy nose and sneezing) of at least 6 within the first two hours of the session. The Stratification of grass pollen allergic subjects into 2 groups will be done according to the severity of their allergic reaction to grass pollen allergens by a combined scoring of reaction in SPT, and reactivity in the pollen chamber.

After screening there will be 3 injection visits at intervals of approximately 4 weeks between each other.

3-4 weeks after the final injection of BM32 subjects will be subjected to SPT and enter the VCC for a 6 hour provocation session.

Each subject will undergo a follow-up visit 7-14 days after the final provocation session.

The total duration of the study for each subject is between 14 and 20 weeks.

Introduction:

1.1 Background

More than 25% of the population suffer from IgE antibody-mediated allergy. The symptoms of allergy include allergic rhinoconjunctivitis, asthma, skin reactions, manifestations in the gastrointestinal tract and severe systemic reactions such as anaphylactic shock. Allergic rhinoconjunctivitis represents the most frequent manifestation of allergy and grass pollen is among the most important allergen sources.

Seasonal allergic rhinitis symptoms are mainly localized at the level of the nose and cause runny nose, bilateral obstruction, sneezing, and nasal pruritus. Frequent eyes symptoms include irritation, lacrimation, and pruritus. Associated symptom complexes can include Eustachian tube dysfunction, asthma and atopic dermatitis. Antagonists of the H1 receptor of histamine have a primary role in controlling the major symptoms of allergic rhinitis and conjunctivitis such as nasal itching, rhinorrhea, sneezing and itchy, watery eyes but such as other symptomatic medications do not represent causative forms of treatment with a disease-modifying effect. Allergen-specific immunotherapy (SIT) is based on the administration of the disease-causing allergens with the aim to induce clinical tolerance to the allergens. It is a causative form of treatment, has disease-modifying effects and may have long lasting effects even after discontinuation. However, the application of SIT is limited because it may cause severe side effects and represents a cumbersome form of treatment requiring time-consuming and inconvenient treatment schedules. Allergy vaccines based on defined and pure recombinant allergen molecules and hypoallergenic allergens derivatives have entered successful clinical studies and hold great promise for the improvement of SIT because they represent defined vaccines which can be designed to reduce side effects.

BM32 is a hypoallergenic grass pollen allergy vaccine for immunotherapy of grass pollen allergy, which consists of an aluminum hydroxide-adsorbed equimolar mix of four active ingredients, BM321, BM322, BM325 and BM326. The four active ingredients are purified recombinant proteins containing non-allergenic peptides from the four major timothy grass pollen allergens, Phl p 1 (BM321), Phl p 2 (BM322), Phl p 5 (BM325) and Phl p 6 (BM326) which are fused to the PreS domain of hepatitis B virus, a protein used in childhood vaccines. The BM32 proteins have been expressed in *Escherichia coli* and were purified under GMP conditions. They do not react with IgE antibodies from allergic patients and show a more than 1000-fold reduced allergenic activity when compared with the complete allergens regarding the activation of basophils from allergic patients. Since the majority of T cell-reactive epitopes of the grass pollen allergens are not present in the BM32 proteins, they should induce only low or no T cell-mediated inflammation. BM32 thus holds promise not to induce IgE mediated immediate type (e.g. anaphylactic reactions) or T-cell mediated late phase side effects during immunotherapy.

“BM32” and its four active ingredients “BM321, BM322, BM325, and BM326” have been named as follows:

- BM: Abbreviation for Biomay
- 3: code number for Biomay´s Peptide/Carrier technology
- 2: code number for indication grass pollen allergy
- Last number in name of APIs (1, 2, 5, or 6): code number for active ingredients (drug substance) refer to the four major grass pollen allergens Phl p 1, Phl p 2, Phl p 5, or Phl p 6.

The active ingredients of BM32 (BM321, BM322, BM325, BM326) are recombinant fusion proteins, each of them consisting of a specific, allergen-derived part (i.e., allergen-derived peptides lacking IgE reactivity) which is fused to a generic carrier protein, the PreS domain of hepatitis B virus which is also part of childhood vaccines

A safety study involving the four components of BM32 and the complete BM32 products in a SPT setting has been completed (study CS-BM32-001). The reactivity to different concentrations of the active components of BM32 and the BM32 mixture has been compared to the reactivity towards a standardized grass pollen extract as well as against histamine. In addition, the propensity of inducing T cell-related late phase reactions was evaluated using atopy patch testing (APT). A total of 60 subjects allergic to grass pollen were included in this study. The expected safety profile was demonstrated, as no relevant allergic reactivity was found for any of the applied concentrations. Patients who demonstrated a positive response to grass pollen extract in the APT, also showed no relevant late phase reactivity to BM 32.

In the planned study CS-BM32-002, the Vienna Challenge Chamber (VCC) will be used together with titrated SPT to evaluate the clinical response of grass pollen allergic individuals treated with subcutaneous injections of BM32 to respiratory exposure to grass pollen.

The VCC allows controlled pollen exposure of allergic patients, for sustained periods. It has been designed to actively manage and reproduce levels of pollen. The VCC is a standardized method for challenging up to 20 subjects at a time with native allergens at standardized concentrations and to obtain repeated reports from each individual. In this way, pollen levels comparable to concentrations observed during corresponding season can be maintained and the course of exposure can be monitored in order to determine whether there is symptomatic variability over the exposure period, which can last for hours. Under such controlled conditions, valid comparisons of anti-allergic medications or monitoring of the clinical effect of SIT is possible.

1.2 Rationale

Specific immunotherapy (SIT) is the only allergen-specific and disease-modifying therapeutic modality for IgE-mediated allergies. However, the current clinical use of extracts from allergy causing agents (pollen, mites, animal hair) is limited by many problems caused by the poor quality of natural allergen extracts, including poor characterization, lack of important allergens and contaminations of the API, inconvenient dosing schemes and occurrence of side effects, in the worst case severe and life-threatening anaphylactic side effects. Due to these problems the use of SIT is limited although it represents a causative and disease-modifying treatment unlike symptomatic pharmacotherapy which only mitigates symptoms. It is therefore highly desirable to develop products for SIT, which overcome these problems and provide a safe and convenient treatment so that a larger number of patients can benefit from this treatment. This promise is approaching reality with improved understanding of the molecular nature of the disease-causing allergens and the disease process itself. Pure allergens can now be obtained by recombinant expression and molecular modifications of the allergens allow developing vaccines with reduced side effects and greater efficacy.

The recombinant proteins constituting the grass pollen vaccine BM32 are the first members of a new generation of allergy vaccines, which have been designed to minimize side effects and to allow establishing a convenient immunotherapy schedule for all major seasonal and perennial allergies. They have been engineered to reduce IgE reactivity and T cell reactivity but to retain the ability to induce upon vaccination protective IgG antibody responses against the natural allergens. Their safety has been studied in allergic patients by skin testing showing almost complete lack of allergenic activity.
This study is designed to investigate safety and dose dependent effects of three subcutaneous injections of BM32 on immunological and clinical responses to grass pollen allergens in patients suffering from grass pollen-induced rhinoconjunctivitis.

Antibody responses to grass pollen allergens will be studied in patients treated with different doses of BM32 or placebo. Clinical effects will be studied by titrated skin prick testing and respiratory exposure to grass pollen allergens.
A standard tool to investigate anti-inflammatory activity of oral or intranasal compounds is the nasal allergen challenge in rhinitis subjects. The traditional single nasal allergen challenge has several problems; a single high dose nasal allergen challenge does not replicate allergen exposure in the environment, it is unpleasant for the subject, it is difficult to compare different trials to each other as allergen doses have to be individually titrated during each study and challenges are time and staff intensive.

The VCC is a more physiological challenge and offers the opportunity to obtain assessments of response from up to 20 subjects in a period of a few hours. Within the VCC, subjects are exposed to sustained low-dose allergen at a pre-determined dose over 4-6 hours. This results in a sustained response, similar to that seen during natural allergen exposure, which rises to a plateau after 90 to 120mins. Results in this model have been shown to be highly reproducible.

Aim of this study is to determine a safe dose of BM32 which affects grass pollen-specific allergic reactions in the skin and upon exposure in the Vienna Challenge Chamber (VCC) and induces immunological changes expected to be associated with clinically effective SIT.

2 Objectives

2.1 Primary Efficacy Objective

- To assess the minimum effective dose after three subcutaneous injections of different dose levels of BM32 as compared to placebo. The effects of different dose levels of BM32 compared to placebo are evaluated by the grass pollen-specific Total Nasal Symptom Score (TNSS – nasal obstruction, rhinorrhoea, itchy nose and sneezing) of grass pollen-induced allergic rhinitis provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection (Visit 8) of the treatment.

2.2 Secondary Efficacy Objective(s)

- To evaluate the effects of different dose levels of BM32 compared to placebo by studying the grass pollen-specific Total Non Nasal Symptom Score (TNNSS), i.e. the Total Ocular Symptom Score (TOSS) (watery eyes, itchy eyes, red eyes) and the Other Symptom Score (OSS) (cough, itchy throat, itchy ears) of grass pollen-induced allergic rhinitis provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection (Visit 8) of the treatment.

- To assess the effects of different dose levels of BM32 compared to placebo, by evaluating the Global Symptom Score (Total Nasal Symptom Score (TNSS) and Total Non Nasal Symptom Score (TNNSS) combined) of grass pollen-induced allergic rhinitis provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection (Visit 8) of the treatment.

- To determine the effects of vaccination with BM32 versus placebo on allergen-specific skin responses by titrated skin prick testing (SPT) via measuring wheal areas at screening and at visit 7 and to evaluate the change in the threshold concentration of grass pollen extract necessary to provoke a positive skin reaction (SPT).

- To evaluate the effects of different dose levels of BM32 compared to placebo by the mean cross-sectional area (MCA) using anterior rhinomanometry (NAR) provoked by spending 6h in the VCC at screening and 6h in the VCC after the last injection of the treatment (Visit 8).

- To evaluate the effects of different dose levels of BM32 compared to placebo by FEV1 (Forced Expiratory Volume in 1 second) and FEV1/FVC (Tiffeneau-Value) as measured during challenge sessions at visit 3 and at visit 8.

2.3 Primary Safety Objective

- To evaluate the relative safety and tolerability of three different dose levels of BM32 compared to placebo.

2.4 Secondary Safety and Immunogenicity Objective(s)

- To determine the effects of vaccination with BM32 versus placebo on allergen-specific antibody responses.

- To determine the effects of vaccination with BM32 versus placebo on allergy related immunological parameters. In particular, antibody subtypes, T-cell responses and cytokine responses to recombinant allergens, grass pollen extract, BM32 components, and the carrier (PreS), as well as the sensitivity to recombinant grass pollen allergens and timothy grass pollen extract in allergen-induced basophile activation and in the CD_{203c} assay will be studied.

3 Endpoints

3.1 Primary Efficacy Endpoint

- Difference in the total Nasal Symptom Score (TNSS) (nasal obstruction, rhinorrhoea, itchy nose and sneezing) between spending 6h in the VCC at screening and spending 6h in the VCC 4 weeks after the last injection of the treatment (Visit 8).

3.2 Secondary Efficacy Endpoints

- Difference in the Total Non-Nasal Symptom Score TNNSS (TOSS and OSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the Global Symptom Score (TNSS and TNNSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the nasal airflow resistance (NAR) (measured using active anterior rhinomanometry) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Change in skin reaction to grass pollen allergens (SPT) before (screening) and after the treatment (visit 7) by dose titration of the grass pollen extract measuring the difference in the sum of wheal areas between these two visits and evaluating the change in the threshold concentration of grass pollen extract necessary to provoke a positive skin reaction (SPT)
- Difference in the FEV1 and FEV1/FVC between Screening and Visit 8

3.3 Primary Safety Endpoint

- Frequency of AEs concerning occurrence, seriousness, intensity and drug-relationship.
- Frequency of local reactions (injection site reactions) and systemic reactions classified by a grading scheme according to the Position Paper of the German Society for Allergology and Clinical Immunology4

3.4 Secondary Safety and Immunogenicity Endpoints

Rescue medication

- Administration of rescue medication via total amount of rescue medication needed.

Vital signs and physical examination

- Blood pressure, pulse rate, body temperature, and breathing frequency, during the whole study period, ECG and physical findings at screening, medication phase, evaluation phase and at the follow-up examination.

[4] Kleine-Tebbe, J. , Fuchs, T. , Klimek, L., et al.: Die spezifische Immuntherapie (Hyposensibilisierung) mit Allergenen. Positionspapier der deutschen Gesellschaft für Allergologie und klinische Immunologie inhaltlich abgestimmt mit dem Ärzteverband Deutsche Allergologen. Pneumologie 2001; 55, pp. 438-44.

Laboratory

- Haematology, biochemistry, and urinalysis at screening, medication phase and at the follow-up examination.
- In women of childbearing potential, a pregnancy test will be performed at screening, medication phase, evaluation phase and at follow-up.

Immunogenicity Endpoints

- Change in allergen specific total IgG levels after 3 s.c. injection with BM32, as measured at screening and visit 7
- Change in allergen specific IgE levels after 3 s.c. injection with BM32, as measured at screening and visit 7
- Change in allergy related immunological parameters (T-cell responses and cytokine responses to recombinant allergens, grass pollen extract, BM32 components, and the carrier (PreS), as well as the sensitivity to recombinant grass pollen allergens and timothy grass pollen extract in allergen-induced basophile activation and in the CD_{203c} assay) levels after 3 s.c. injection with BM32, as measured in blood or serum samples collected at screening (Baseline) and after the last injection of the treatment (visit 7).

4 Study Design

This will be a prospective, randomized, double-blind, placebo-controlled, parallel group, safety and dose-finding (concerning minimum effective dose (MED) study to evaluate the safety and tolerability of BM32 grass pollen vaccine compared to placebo and to study its effects on clinical responses to grass pollen allergen challenge and immunological responses to vaccination.
There will be 4 study groups: 3 dose levels of BM32, and placebo.

- Screening (Visit 1-3) – Physical examination, anamnesis, blood sampling, determination of allergen-specific IgE levels, skin prick test titration and determine eligibility, including having an adequate, measurable nasal allergic response upon exposure to grass pollen in the Vienna Challenge Chamber (VCC) and randomization to the different study groups
- Washout – ≥ 3 days
- Visit 4 – first injection of BM32 or placebo
- 4 week break (- 3/ + 7 days)
- Visit 5 –, second injection of BM32 or placebo
- 4 week break (- 3/ + 7 days)
- Visit 6 –third injection of BM32 or placebo
- 3 week break (+ 7 days)
- Visit 7 – blood sampling and titrated Skin Prick Test
- Visit 8 – 6 h provocation session (0 -7 days after Visit 7)
- Follow-up (Visit 9) – ≥ 7 days After Visit 8

5 Study Population

The target number of subjects for this study is 60. To allow for drop outs this study will include up to 72 male and female subjects, aged 18 to 60 years with a clinical history of SAR during each of the last two grass allergy seasons, and a positive skin prick test to grass allergen within 12 months prior to Visit 1.

6 Eligibility Criteria

6.1 Inclusion Criteria

A subject will be eligible for inclusion in this study only if all of the following criteria apply:

- The subject is allergic but otherwise healthy. Healthy subjects are defined as individuals who are free from clinically significant illness or disease as determined by their medical history (including family), physical examination, laboratory studies, and other tests deemed by the Investigator or designee..
- Male or female between 18 and 60 years of age inclusive, at the time of signing the informed consent. They have a history of seasonal allergic rhinitis (SAR) to grass pollen.
- They have a normal electrocardiogram without clinically significant abnormalities deemed by the Investigator or designee.
- They exhibit a moderate to severe response to approximately 1500 grass pollen grains/m3 after the first 2h in the Vienna Challenge Chamber, which is defined as a nasal symptom Score (TNSS) of at least 6. (Nasal symptom Score is the sum of nasal obstruction, rhinorrhoea, itchy nose and sneezing, each of which have been Scored on a scale from 0 to 3).
- They have a positive skin prick test (wheal diameter $\geq$ 3mm) for grass pollen at or within 12 months preceding the screening visit.
- They have a positive RAST (class $\geq$ 2) for timothy grass pollen (g6) and to rPhl p 1+rPhl p 5 at or within 12 months preceding the screening visit.
- There are no conditions or factors which would make the subject unlikely to be able to stay in the chamber for 6 hours.
- They are capable of giving informed consent which includes compliance with the requirements and restrictions listed in the consent form.
- They are available to complete all study measurements.

6.2 Exclusion Criteria

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

- Pregnant, lactating or sexually active women with childbearing potential who are not using a medically accepted birth control method (pregnancy to be controlled by a pregnancy dipstick test).
- On examination the subject is found to have any structural nasal abnormalities or nasal polyposis, a history of frequent nosebleeds, recent nasal surgery or ongoing upper respiratory tract infection which in the Responsible Physician's opinion renders the subject unsuitable for participation in the study.
- Any respiratory disease other than mild stable asthma that is controlled with occasional use of as-needed short-acting beta-agonists and associated with normal lung function.
- The subject is concurrently participating or has participated in any clinical study in the previous month.
- The subject has received SIT for grass pollen allergy in the last two years prior to this study.
- Past or present disease, which as judged by the investigator, may affect the outcome of this study. These diseases include, but are not limited to, cardiovascular disease, malignancy, hepatic disease, renal disease, haematological disease, neurological disease, endocrine disease or pulmonary

disease (including but not confined to chronic bronchitis, emphysema, bronchiectasis or pulmonary fibrosis).

- autoimmune diseases, immune defects including immuno-suppression, immune-complex-induced immunopathies
- Suspected hypersensitivity to any ingredients of the study medication
- Use of prohibited medication prior to Screening and throughout the study:
  - Depot corticosteroids – 12 weeks
  - Oral corticosteroids – 8 weeks
  - Inhaled corticosteroids – 4 weeks
- The subject is at risk of non-compliance with the study procedures/restrictions.
- Allergic symptoms at the time of screening
- Any other reason that the Investigator considers makes the subject unsuitable to participate.

7 Study assessments and procedures

7.1 Subject identifier

Each subject will be identified by initials (only letters, without spaces or any punctuation signs like coma, slash, hyphen …) and subject identifier, including Screening number and subject number according to the randomisation.

7.2 Assessment of Demographic Data

Demographic data recorded as part of the pre-study screening assessment will be captured electronically and entered into the study CRF. Having given informed consent volunteers will be required to undergo a medical screen 7-28 days prior to the first dosing occasion to determine whether they are eligible to participate in the study according to the inclusion/exclusion criteria.

7.3 Screening, Inclusion, Immunologic & Baseline assessments (Visit 1-3)

The screening visit, as described in the Time and Events Schedule (Appendix 1), will include:

7.3.1 Visit 1 – Pre-Screening

- Signing of the Informed Consent Form
- Demographic data recording
- Full medical history
- Full Physical examination
- Vital signs
- Pulmonary function test
- 12-lead Electrocardiogram
- Pregnancy test for females
- Concomitant medication recording
- Blood and urine sampling for clinical laboratory assessment (Haematology, Clinical chemistry and Serology as well as Urinalysis)
- SME assessment

7.3.2 Visit 2 – SPT & Immunology (Baseline)

- Titrated skin prick test for grass pollen
- Blood sampling for assessment of immunological parameters
- Vital signs
- Concomitant medication recording
- SME assessment

7.3.3 Visit 3 – VCC Baseline

- Baseline VCC challenge session (subjective scoring, rhinomanometry, nasal secretion, spirometry) to ensure subjects exhibit a moderate response to approximately 1,500 grass pollen grains/m3 after 2 hours provocation. Total duration of the session: 6 h
- Pulmonary function test
- Vital signs
- Concomitant medication recording
- SME assessment

7.4 Randomization procedure

Randomization will be carried out as block randomization stratified for allergy severity (two groups: 1. moderate, 2. severe) as measured by a composit index from titrated SPT, and TNSS at the qualification challenge session.The detailed stratification criteria are described in a separate study procedure titled “KA-GCP-003 Randomisation and Stratification_CS-BM32-002” which will be included in the SRM

7.5 Treatment period

7.5.1 Visit 4 - Randomization & first injection

After a Washout of at least 3 days after the baseline provocation session, subjects come back to the VCC for the first administration of IMP:

- On arrival to the unit subjects will be asked about their state of health and the use of any concomitant medication details of which will be recorded in the CRF
- Brief physical examination
- Pregnancy test for females
- Vital signs prior and 15-30min after injection of IMP
- Pulmonary function test
- 12-lead Electrocardiogram prior and 15-30min after injection of IMP
- IMP administration: s.c. injection of different dose levels of BM32 or Placebo
- Blood and urine sampling for clinical lab safety assessment 15-30min after IMP administration
- AE recording
- Instructions for concomitant medication & AE sheet
- At least 30 minutes medical observation period of subjects

7.5.2 Visit 5 – second injection

4 weeks (- 3 days/ + 7 days) after the first injection, subjects come back to VCC.

- Concomitant medication recording
- AE recording
- Pregnancy test for females
- Brief physical examination
- Vital signs prior and 15-30min after injection of IMP
- Pulmonary function test
- Blood and urine sampling for clinical lab safety assessment 15-30min after IMP administration
- 12-lead Electrocardiogram prior and 15-30min after injection of IMP
- IMP administration: s.c. injection of different dose levels of BM32 or Placebo
- Instructions for concomitant medication & AE sheet
- At least 30 minutes medical observation period of subjects

7.5.3 Visit 6 – third injection

4 weeks (-3 days/+7 days) after the second injection, subjects come back to VCC.

- Concomitant medication recording
- AE recording
- Pregnancy test for females
- Brief physical examination
- Vital signs prior and 15-30min after injection of IMP
- Pulmonary function test
- Blood and urine sampling for clinical lab safety assessment 15-30min after IMP administration
- 12-lead Electrocardiogram prior and 15-30min after injection of IMP
- IMP administration: s.c. injection of different dose levels of BM32 or Placebo
- Instructions for concomitant medication & AE sheet
- At least 30 minutes medical observation period of subjects

7.5.4 Visit 7– SPT and Immunological assessments

3 weeks (+7 days) after the third and last injection, subjects come back to the facility for assessment of the sensitivity to grass pollen by titrated skin prick test and immunological assessments

- Concomitant medication recording
- AE recording
- Brief physical examination
- Vital signs
- Blood sampling for assessment of immunological parameters
- Titrated skin prick test for grass pollen
- Pregnancy test for females

7.5.5 Visit 8 – Provocation session in the VCC

Up to 7 days after Visit 7 subjects will come back to VCC for the 6-hour provocation session.

- Concomitant medication recording
- AE recording
- Vital signs
- Pregnancy test for females
- Pulmonary function test
- Baseline assessments (Subjective Scoring, rhinomanometry, spirometry)
- 6 hour provocation session in the Vienna Challenge Chamber
- 12-lead Electrocardiogram after the challenge

7.6 Follow up (Visit 9)

7-14 days after Visit 8 the subjects will come back to the facility for the follow up assessments as follows:

- Full physical examination
- Concomitant medication recording
- AE recording
- Pregnancy test for females
- Vital signs
- Pulmonary function test
- Blood and urine sampling for clinical lab safety assessment
- 12-lead Electrocardiogram

7.7 Safety Laboratory Monitoring

Samples collected will be analysed at a local laboratory. Whole blood and urine samples will be collected and processed according to the local procedures at site. The samples will then be transferred to the local laboratory for analysis.

Hematology, clinical chemistry and urinalysis (done by Accu-Tell®) will be conducted at screening, after administration of study medication, before the final challenge, and at follow-up.

7.8 Vital Signs (PR, BP, T, BF)

Vital sign measurements of pulse rate (PR), blood pressure (BP), temperature (T) and breathing frequency (BF) will be monitored throughout the whole study during every visit as well as pre and post challenge.
Vital signs measurements will be made with the subject in a sitting position having rested in this position for at least 3 minutes before the first reading at each time point.
Measurements that deviate substantially from previous readings will be repeated immediately. If any results falling outside of the normal ranges are deemed clinically significant by the investigator or appropriately qualified designee then these should be recorded in the CRF as an AE/SAE (see Section 10).

7.9 12-lead electrocardiogram (ECG)

The ECG measurements will be made with the subject in a supine position. Computerised 12-lead ECG recordings will be obtained. Each lead shall be recorded for at least 3-5 beats at a speed of 25 mm/sec. The following parameters will be recorded: rhythm, ventricular rate, PQ interval, QRS duration, QT and QTc (see sample-ECG in the appendix section).
The correction of the QT interval will be calculated in accordance with the formula of Bazett (QTc = QT-interval/√RR-distance).
Any results falling outside the normal ranges may be repeated at the discretion of the investigator. If any results falling outside of the normal ranges are deemed not clinically significant by the investigator or appropriately qualified designee, then this should be clearly stated on the hard copies of the ECG and signed and dated by the investigator. If the ECG trace indicates an abnormality that is measured by the equipment but is deemed normal by the investigator then this should be clearly stated on the ECG trace as normal and signed and dated by the investigator or appropriately qualified designee. If the ECG trace indicates an abnormality that is present but deemed as not clinically significant by the investigator or appropriately qualified designee then this should be clearly stated on the ECG trace as “NCS” and signed and dated by the investigator or appropriately qualified designee. If any results falling outside of the normal ranges are deemed clinically significant by the investigator or appropriately qualified designee then these should be recorded in the CRF as an AE/SAE (see Section 10).
ECG’s will be stored electronically for manual measurement of intervals, if necessary.

7.10 Pregnancy

7.10.1 Time period for collecting pregnancy information

Females of childbearing potential will be tested for pregnancy by serum or urine methods at least every 28 days throughout the study (specified in the time & event schedule – appendix). If timely suitable this will be done during Screening (Visit 1-3), Visit 4 to 6 (prior to IMP administration), at Visit 8 ahead of the provocation start and at the follow-up visit (Visit9). In the case of a positive serum or urine β-hCG test the subject will be withdrawn from the study.

7.10.2 Action to be taken if pregnancy occurs

The investigator will collect pregnancy information on any female subject, who becomes pregnant while participating in this study. The investigator will record pregnancy information on the appropriate form and submit it to the Sponsor upon learning of a subject's pregnancy. The subject will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE (see AE/SAE section of the protocol).
A spontaneous abortion is always considered to be an SAE and will be reported as such.

Furthermore, any SAE occurring as a result of a post-study pregnancy and is considered reasonably related to the investigational product by the investigator, will be reported to the Sponsor as described in the AE/SAE section. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

7.11 Efficacy & Safety Assessments

7.11.1 Skin Prick Test and RAST test

Skin prick tests and RAST tests will be performed at screening (if not done in the previous 12 month) to determine eligibility if subjects have not had positive results in the 12 months prior to screening in accordance with the site SOP. Further details are provided in the SRM.

7.11.2 Assessment of immunological parameters

Blood samples for the assessment of immunologic parameters will be collected at visit 2 and visit 7. Blood samples will be processed and analyses will be performed at external labs at the Medical University of Vienna. In total, 50 mL of heparinized whole blood and 50 mL in serum vials are required per blood collection. Details on the individual immunological asssssments are provided in the SRM.

Samples from each time point will be frozen and stored as aliquots at –80°C for further analyses, if necessary.

Measurement of allergen-specific antibody levels in serum

Allergen-specific IgG and IgE antibody levels will be measured by ELISA in each of the obtained serum samples as described (Niederberger et al., Proc. Natl. Acad. Sci. USA 2004; Pree et al., J. Immunol. 2006). Allergen-specific IgG will be evaluated with respect to their ability to block binding of grass pollen allergen-specific IgE to grass pollen allergens.

Additionally, grass pollen allergen specific IgG and IgE levels and will be determined using the ImmunoCAP system (Phadia, SE) and sensitization profiles (assessment of specific IgE to a variety of allergens) will be determined using the ImmunoCAP ISAC mulitplex assay (Phadia, SE). Assays will be performed according to the manufacturer's instructions.

Measurement of carrier-specific antibody levels in serum

PreS-specific IgG and IgE antibody levels will be measured by ELISA in each of the obtained serum samples as described (Niespodziana et al., J. Clin Immunol. 2011).

CD203c assay for assessment of the allergic sensitivity to grass pollen allergens

The CD203c assay has been proposed as a potential surrogate parameter for the efficacy of SIT. Basophils from heparinized blood samples are exposed to different concentrations of equimolar mixtures of recombinant grass pollen allergens Phl p 1, Pl p 2, Pl p 5, and Phl p 6, equimolar mixtures of the active components of BM32, and anti-IgE as positive control. Up-regulation of CD203c expression on the basophils is detected by FACS.

T-cell proliferation assay

Peripheral blood mononuclear cells (PBMC) are isolated from heparinised blood samples by Ficoll (Amersham Pharmacia Biotech, Little Chalfont, UK) density gradient centrifugation, and cultured at 37°C. Cells are stimulated with different concentrations of BM32 active components (equimolar mixture), equimolar concentrations of synthetic peptides, PreS, recombinant grass pollen allergens, and grass pollen extract. The cell proliferation is detected via incorporation of [3H]thymidine by liquid scintillation counting.

Cytokine profiles

The cytokine expression of T-cells (PBMCs isolated from heparinized blood samples) after activation with mixtures of recombinant grass pollen allergens, active components of BM32, or PreS will be determined from the supernatants of PBMC cultures using the xMAP derived Bio-Plex (BIO-RAD, Hercules CA, USA) fluorescent bead-based technology. This system allows simultaneous testing of 17 human cytokines, chemokines, and growth factors (IL-1ß, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, MCP1, MIP 1ß, IFN-g, and TNF-a).

7.11.3 Titrated Skin Prick Test

A skin prick test titration will be performed at Screening and during Visit 7. A SOP describing the test procedure in detail will be provided by the sponsor. Briefly, a registered standardized timothy grass pollen extract (Stallergenes, Antony, France) will be diluted in 1:2 steps and the threshold concentration necessary to elicit a positive skin reaction is determined as described (Drachenberg et al., Allergy 2001; 56, 498-505). Further details are provided in the SRM.

7.11.4 Total Nasal Symptom Score (TNSS)

The TNSS consist of four areas - the nasal obstruction, rhinorrhoea, itchy nose and sneezing. Each of them will be scored before the challenge and then every 15 minutes up to 6 hours (Screening) or 6 hours (Visit 8) on a categorical scale from 0 to 3 at the times described in the Time and Events Schedule.

The categorical scale of symptoms will be defined as follows:

0	None	No symptoms of disease
1	Mild	Signs of disease/ symptoms present from time to time, easily being tolerated
2	Moderate	Signs of disease/ symptoms always present, disturbing, but can be tolerated
3	Severe	Signs of disease/ symptoms always present, can hardly be tolerated

The total TNSS Score is the sum of all 4 symptom categories ranging from 0 to a maximum of 12.

7.11.5 Total Non-Nasal Symptom Score TNNSS: Total Ocular Symptom Score (TOSS) and Other Symptoms Score (OSS)

Three areas will be evaluated for the Total Ocular Symptoms Score (TOSS): watery eyes, itchy eyes and red eyes. By the Other Symptom Score (OSS) three areas will be analysed: cough, itchy throat, itchy ears. They both will be scored before the challenge and then every 15 minutes up to 6 hours (screening) or 6 hours (Visit 8) on a categorical scale from 0 to 3 at the times described in the Time and Events Schedule.

The categorical scale of symptoms will be defined as follows:

0	None	No symptoms of disease
1	Mild	Signs of disease/ symptoms present from time to time, easily being tolerated
2	Moderate	Signs of disease/ symptoms always present, disturbing, but can be tolerated
3	Severe	Signs of disease/ symptoms always present, can hardly be tolerated

The total TOSS Score is the sum of all 3 symptom categories ranging from 0 to a maximum of 9.

The total OSS Score is the sum of all 3 symptom categories ranging from 0 to a maximum of 9.

The TNNSS is calculated by the sum of the TOSS and OSS Score.

7.11.6 Global Symptom Score (TNSS and TNNSS)

The Global Symptom Score will be calculated from the sum of the Total Nasal Symptom Score (TNSS) and the Total Non-Nasal Symptom Score (TNNSS).

7.11.7 Nasal Airflow Resistance

Nasal airflow resistance will be measured every 30 minutes using active anterior rhinomanometry on challenge day at the times described in the Time and Events Schedule (Appendix 1). Further details are provided in the SRM.

7.11.8 Pulmonary function test (PFT)

FEV1 will be measured at the time points specified in the Time and Events Schedule (Appendix 1). Further details are provided in the SRM.

7.11.9 ECGs

12-lead ECG recordings (sample see Appendix 2) will be made at the times described in the Time and Events Schedule (Appendix 1). Further details are provided in the SRM.

7.11.10 Safety Lab

Haematology, clinical chemistry, urinalysis and additional parameters (Serology at Screening only) will be tested at the time-points described in the Time & Event schedule (Appendix 1). Further details of the analysis parameters are provided in Appendix 3.

7.11.11 Vital signs

Vital sign measurements will be taken at time points stipulated in the Time and Events tables (Appendix 1) Details are provided in the Study Reference Manual (SRM).

7.12 Concomitant medication and AE recordings

Subjects will be asked about the use of any medication since their last visit. Details of any medications taken will be recorded in the CRF on the concomitant medication page (for details see chapter 9). AEs are recorded according to chapter 11.

7.13 VCC provocation session (Visit 3, Visit 8)

- Vital Signs (pulse rate, blood pressure, temperature and breathing frequency) pre- and post challenge
- Total Nasal Symptom Score (TNSS - obstruction, rhinorrhoea, itch, sneeze) Scored on a categorical scale from 0 to 3 pre-challenge, every 15mins from 0 to 6h post-start of challenge
- Total Ocular Symptom Score (TOSS) (red eyes, itchy eyes, watery eyes) Scored on a categorical scale from 0 to 3 pre-challenge, every 15mins from 0 to 6h post-start of challenge
- Total Other Symptoms (OSS) (cough, itchy throat, itchy ears) Scored on a categorical scale from 0 to 3 pre-challenge and every 15mins from 0 to 6h post-start of challenge
- Active anterior rhinomanometry (nasal airflow resistance) pre-challenge and every 30mins from 0 to 6h post-start of challenge
- Secretion weight (wet tissue weight) every 30mins from 0 to 6h post-start of challenge
- FEV1 and FVC will be measured pre-challenge and every hour from 0 to 6h post-start of challenge

8 Investigational Product(s)

8.1 Description of Investigational Products

BM32 is a vaccine for specific immunotherapy (SIT) of grass pollen allergy. In contrast to vaccines for SIT of grass pollen allergy available on the market or currently under development, BM32 has been designed to exclusively elicit blocking IgG type antibodies against grass pollen allergens, while eliminating both types of therapy-related side effects observed in SIT treatment: IgE mediated immediate type (e.g. anaphylactic reactions) as well as T-cell mediated late phase side effects. For this reason, it is expected that BM32 will dramatically simplify the procedure of SIT as it is practiced currently. The target is to substantially reduce the number of annual injections, and to make SIT similar to standard vaccination procedures.

BM32 is an equimolar combination of four active ingredients (APIs) which are referred to as “BM321, BM322, BM325, and BM326”. The APIs are adsorbed on aluminum hydroxide as an adjuvant in a physiological buffer containing 0.9% NaCl.

The placebo preparation contains the adjuvans (aluminum hydroxide) alone in the same buffer.

8.1.1 Dose forms and strengths

Manufacture of the IMPs: Polymun Scientific GmbH, Vienna (formulation and aseptic filling of IMPs)
Biomay AG, Vienna (manufacture of APIs; quality control and release of IMPs)

BM32 and Placebo are provided as suspensions “ready for injection”.

In order to allow the blinded administration of three different dose levels, BM32 has been produced in three different strengths (S1, S2, and S3), containing 10µg, 20µg, and 40µg of each of the 4 APIs in the intended injection volume of 400µL, respectively. The specifications of BM32 and Placebo with respect to content of APIs and aluminum hydroxide are summarized in Table 1.

Table 1. Specifications of different strengths of BM32 and Placebo.

	BM32 Drug Products & Placebo			
	BM32/S1	**BM32/S2**	**BM32/S3**	**BM32 Placebo**
Lot no. (blinded)	BM32C-VAC-1101	BM32B-VAC-1101	BM32A-VAC-1101	BM32P-VAC-1101
Concentration BM32 (total sum of all 4 APIs BM321, BM322, BM325, BM326) [mg/mL]	0.1 mg / mL	0.2 mg / mL	0.4 mg / mL	0.0 mg / mL
Dose of each individual BM32 API per 400µL injection	**10 µg**	**20 µg**	**40 µg**	**0 µg**
Concentration aluminum hydroxide [mgAl^{3+}/mL]	0.75 mg / mL	1.5 mg / mL	3 mg / mL	3 mg / mL
Aluminum hydroxide per 400µL dose	0.3 mg	0.6 mg	1.2 mg	1.2 mg
Filling volume per vial	550 µL	550 µL	550 µL	550 µL
Storage	2-8°C	2-8°C	2-8°C	2-8°C

8.2 Dosing schedule

The dosing schedule consists of 3 subcutaneous injections of 400µL BM32/S1, BM32/S2, BM32/S3, or Placebo administered with intervals of 4 weeks (-3 days / + 7 days).

8.3 Packaging

IMPs (BM32 and Placebo) are provided in sterile 2 mL borosilicate glass vials (Afton, RTF8422) with elastomeric stoppers and metal seals. Each vial contains 550µL to allow sufficient extraction of a single 400 µL dose of IMP.

Vials intended for treatment of one individual (i.e. always 3 vials of either BM32/S1, BM32/S2, BM32/S3, or Placebo) will be pooled in paperboard subject boxes.

8.4 Labelling and Blinding

Labelling and blinding of the IMP for the study will be performed at ABF Pharmaceutical Services GmbH, A-1120 Wien.

Each treatment vial as well as each subject box will be labelled to ensure a clear and explicit identification. The label will not contain any information on the strength of the BM32 vaccine or nature of the IMP (active substance or placebo). Instead, each vial will be assigned an identification number according to a predefined random list.

The labels will be designed to meet all local regulatory requirements and match the size of the investigational package.

8.5 Investigational Product Accountability

The investigator is responsible for investigational product accountability and record maintenance. In accordance with all applicable regulatory requirements, the investigator or designated site staff must maintain investigational product accountability records throughout the course of the study. This person(s) will document the amount of investigational product received from the sponsor and the amount supplied and/or administered to subjects.

The investigator will receive numbered treatment boxes (at least 60pcs.) in a clear chronological order and the matching emergency blinding codes in a sealed envelope for storage.
The treatments will be assigned to eligible subjects according to the ascending order of their enrolment.

The investigational drug accountability log form contains at least following information (see sample in Appendix):

- Screening Number
- Randomisation Number (= Treatment number)
- Date of randomisation
- Initials of the subjects
- Responsible site staff member for administration

After completion of the trial, all allotted (including empty containers) and not allotted investigational product containers must be returned to the Sponsor, preferably in their original package.

8.6 Maintenance of Trial Treatment Randomization Codes and Procedures for Blind breaking

The randomization codes of investigational products (identity of the investigational products) will be contained in sealed envelopes, one for each subject, carrying a label with the protocol number, treatment and period number, and Investigator's name, with the instruction: *"To be opened only in case of an emergency".*

The Sponsor should preferably be consulted prior to the blind being broken, but in any case, unblinding must be reported to the Sponsor immediately. If any patient treatment is unblinded, the date and reason for breaking the blind and the person doing so must be reported on the envelope with Investigator's date and signature and in the appropriate CRF page.

These envelopes should be kept in a secure place with restricted access and returned to the Sponsor at the end of the trial.

8.7 Handling and Storage of IMP

A detailed specification of storage conditions as well as instructions regarding handling and in-use stability of the study medication is provided by the sponsor in a separate study procedure titled "KA-GCP-002 Storage and handling of BM32 vaccine and placebo" which will be included in the SRM. In short, the IMP should be stored at refrigerated temperatures; it is important that it is not frozen.

Investigational product must be administered according to procedures described herein.
Only subjects enrolled in the study may receive investigational product, in accordance with all applicable regulatory requirements.
Only authorized site staff may supply or administer investigational product. All investigational products must be stored in a secure area with access limited to the investigator and authorized site staff and under physical conditions that are consistent with investigational product-specific requirements.

The Site will report a climatic condition storage log file to ensure the storage necessities of the investigational drug and to meet the Sponsors storage conditions.

9 Concomitant Medications and Non-Drug Therapies

9.1 Rescue medication

Rescue medication is any medication needed to alleviate anaphylactic reactions occuring as a result of IMP administration. Details and dose of any rescue medication needed should be recorded in the CRF and its use attributed to "rescue medication" – or not, as appropriate, on the concurrent medication page.

9.2 Stand-by medication

Occasional use of inhaled short-acting beta-agonists is permitted as rescue medication if discontinued 24 hours prior to subsequent visits. Subjects may use antihistamines and/or decongestants during the washout/intermediate periods up to 96 and 24 hours prior to the subsequent visit, respectively. Details of any stand-by medication needed should be recorded in the CRF and its use attributed to "stand-by medication" – or not, as appropriate, on the concurrent medication page.

9.3 Concurrent Medications

During the study, concurrent medication will only be given if deemed strictly necessary by the Investigator. All concurrent medications will be recorded in the CRF with their daily dosage, duration and reasons for administration. Subjects who have received any concurrent treatment may be withdrawn from the study at the discretion of the Investigator

10 Subject Completion and Withdrawal

10.1 Subject Completion

Subjects will be considered to be evaluable when they have completed all visits satisfactorily. Non-completers by this definition should be reported and documented in the drug accountability log file. Non-completers may still have their data included in the analysis if they meet inclusion criteria specified in Section 6.

10.2 Subject Withdrawal

10.2.1 Subject Withdrawal from Study

A subject may voluntarily discontinue participation in this study at any time. The investigator may also, at his or her discretion, discontinue the subject from participating in this study at any time. If a subject is prematurely discontinued from participation in the study for any reason, the investigator must make every effort to perform all the evaluations detailed in the follow up visit. These data should be recorded.

10.2.2 Stopping Criteria

Individual subjects will be withdrawn based on a complete evaluation of the results of their safety assessments and their tolerability profile as judged by the respective adverse event reporting profile.

Dose administration may also be halted at the discretion of the investigator based on safety/tolerability data.

Subjects who withdraw or are withdrawn from the study will not be replaced.

10.2.3 Subject Withdrawal from Investigational Product

Subjects will be considered to have prematurely discontinued the study drug if they do not complete all their treatment occasions for any reason.
Once a subject has discontinued study drug, the subject may not re-enter the study. Dosing of the subjects with study medication will be stopped at any time, at the request of the subject, or at the discretion of the principal Investigator (i.e. if clinically significant adverse events should occur).

10.3 Screen and Baseline Failures

Any subject who signs the informed consent but is for any reason not randomised into the study will be considered a screening failure. The screening pages of the CRF's for these subjects will not be filled in but the data will be available to the monitor at the site during the study.

11 Adverse Events (AE) and Serious Adverse Events (SAE)

Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an adverse event includes following:

- Significant or unexpected worsening or exacerbation of the condition/indication under study. ("Lack of Efficacy")
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Significant failure of expected pharmacological or biological action.

Examples of an AE **does not include** a/an:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure are an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.

11.1 Definition of a SAE

A serious adverse event is any untoward medical occurrence that, at any dose:

a) Results in death.

b) Is life-threatening.

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c) Requires hospitalization or prolongation of existing hospitalization.

NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in disability/incapacity, or

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly/birth defect.

f) Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

11.2 Lack of Efficacy

"Lack of efficacy" per se will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the AE or SAE definition (including clarifications).

11.3 Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs and SAEs

Abnormal laboratory findings (e.g., clinical chemistry, haematology) or other abnormal assessments (e.g., FEV1, ECGs, vital signs, etc.) that are judged by the investigator as **clinically significant** will be recorded as AEs or SAEs if they meet the definition of an AE, as defined in Section 10.1 ("Definition of an AE"), or SAE, as defined in Section 10.2. ("Definition of a SAE").

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will **not** be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

11.4 Time Period, Frequency, and Method of Detecting SAEs/ AEs and Serious Medical Events (SME)

Events related to study participation during the screening period (i.e. before administration of the investigational product) will be recorded as Serious Medical Events (SME). AEs and SAEs will be recorded between receipt of first dose and the follow-up visit.
Each subject will be monitored by the Investigator or study personnel for adverse events occurring throughout the study. During the treatment period, the Investigator or designee will inquire about AEs by asking the following standard questions:

At the first scheduled AE enquiry on each study day (pre-dose) subjects will be asked:
"How are you feeling?"

At subsequent scheduled intervals, see Time and Events Schedule (Appendix 1), subjects will be asked:
"Since you were last asked, have you felt unwell or different from usual?"

11.5 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the CRF. It is not acceptable for the investigator to send photocopies of the subject's medical records to THE SPONSOR in lieu of completion of the appropriate AE/SAE CRF pages. However, there may be instances when copies of medical records for certain cases are requested by THE SPONSOR. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to THE SPONSOR.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

11.6 Evaluating AEs and SAEs

11.6.1 Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the investigator's clinical judgement. The intensity of each AE and SAE recorded in the CRF should be assigned to one of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.
- Severe: An event that prevents normal everyday activities.

An AE that is assessed as severe should not be confused with a SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 10.2., "Definition of a SAE".

11.6.2 Assessment of Causality

The investigator is obligated to assess the relationship between investigational product and the occurrence of each AE/SAE. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the CIB/IB and/or Product Information, for marketed products, in the determination of his/her assessment.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to THE SPONSOR. However, it is very important that the investigator always make an assessment of causality for every event prior to transmission of the SAE CRF to THE SPONSOR. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE CRF accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

The investigator will provide the assessment of causality as per instructions on the SAE form in the CRF.

11.7 Follow-Up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide further information to the Sponsor on the subject's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs and SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE/SAE CRF page(s) will be updated. The investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE.

This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

The Sponsor may request that the investigator perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obligated to assist. If a subject dies during participation in the study or during a recognized follow-up period, THE SPONSOR will be provided with a copy of any post-mortem findings, including histopathology.

New or updated information will be recorded on the originally completed "SAE" CRF, with all changes signed and dated by the investigator. The updated SAE CRF should be resent to SPONSOR within the time frames given in section 10.9.1.

11.8 Prompt Reporting of SAEs to the Sponsor

SAEs will be reported promptly to THE SPONSOR as described in the following table once the investigator determines that the event meets the protocol definition of an SAE.

11.8.1 Timeframes for Submitting SAE Reports to Sponsor

	Initial SAE Reports		Follow-up Information on a Previously Reported SAE	
Type of SAE	**Time Frame**	**Documents**	**Time Frame**	**Documents**
All SAEs	24 hrs	"SAE" CRF pages	24 hrs	Updated "SAE" CRF pages

11.8.2 Completion and Transmission of the SAE Reports

Once an investigator becomes aware that an SAE has occurred in a study subject, she/he will report the information to THE SPONSOR within 24 hours. The SAE CRF will always be completed as thoroughly as possible with all available details of the event, signed by the investigator (or designee), and forwarded to THE SPONSOR within the designated time frames. If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying THE SPONSOR of the event and completing the form. The form will be updated when additional information is received.
The investigator will always provide an assessment of causality at the time of the initial report as described in Section 11.6.2., "Assessment of Causality".

Facsimile transmission of the "SAE" CRF is the preferred method to transmit this information to the project contact for SAE receipt. In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the "SAE" CRF sent by overnight mail. Initial notification via the

telephone does not replace the need for the investigator to complete and sign the SAE CRF within the time frames outlined in Section 11.8.1, “Timeframes for Submitting SAE reports to Sponsor”.

THE SPONSOR will provide a list of project contacts for SAE receipt, fax numbers, telephone numbers, and mailing addresses.

11.9 Post-study AEs and SAEs

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE detection period defined in Section 11.4., “Time Period, Frequency, and Method of Detecting AEs and SAEs”, of the protocol.

Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the investigator will promptly notify THE SPONSOR.

11.10 Serious Medical Events (SME)

An event considered related to study participation (e.g., procedures, invasive tests, a change in existing therapy) and occurring during the screening period (i.e. before administration of the investigational product) will be recorded as Serious Medical Event (SME), and has to be reported promptly to THE SPONSOR

12 Data Management and Statistics

12.1 Data management and quality control

12.1.1 Case report forms

The efficacy data collected in the VCC chamber will be captured electronically and excerpted from the database system. All data not available in digital form will be collected on paper CRFs .

Compound: BM32	**Study Number: 3007**
Principal Investigator:	Univ.Prof.Dr. F. Horak
Abbreviated Site Address:	Vienna Challenge Chamber, Vienna, Austria
DATA Type or Item	**Source DOCUMENT *** * (for example, written subject notes, nurses notes, intensive care unit chart, computerised medical records, diary card, health outcome questionnaire, study worksheet, drug accountability log, CRF, laboratory report, specific diagnostic test results such as ECG traces, spirometry reports, etc.)
Medical History	Patient master data sheet – Volunteer folder
Physical examination	Physical examination sheet – additional examinations folder
Demographics	Workbook – Volunteer folder
Inclusion/Exclusion criteria	Workbook – Volunteer folder
RAST & SPT	Patient Master data sheet – Volunteer folder
SPT	Study specific SPT sheet - additional examinations folder
ECGs	Signed ECG printout – additional examinations folder
Vital Signs	Signed Vital signs sheet printout – Study folder
FEV1	Digital Excel spreadsheet – Allover file
TNSS	Digital Excel spreadsheet – Allover file
Total Ocular Symptom Scores	Digital Excel spreadsheet – Allover file
Other Symptom Scores	Digital Excel spreadsheet – Allover file
Rhinomanometry	Digital Excel spreadsheet – Allover file
Secretion weight	Digital Excel spreadsheet – Allover file
AEs	CRF - Volunteer folder
SAEs	CRF - Volunteer folder
Concomitant medication	CRF - concomitant med page
Study drug injection	CRF & daily checklist – Study folder
Laboratory safety results	Signed Lab printout – Laboratory folder
Immunogenicity Data	Signed report – paper printout

Compound: BM32	**Study Number: 3007**
Principal Investigator:	Univ.Prof.Dr. F. Horak
Abbreviated Site Address:	Vienna Challenge Chamber, Vienna, Austria
DATA Type or Item	**Source DOCUMENT *** * (for example, written subject notes, nurses notes, intensive care unit chart, computerised medical records, diary card, health outcome questionnaire, study worksheet, drug accountability log, CRF, laboratory report, specific diagnostic test results such as ECG traces, spirometry reports, etc.)
Pregnancy Test	Urine pregnancy test – Workbook; Urinalysis sheet - Laboratory folder

All the information collected during the study will be recorded in the case report forms (CRFs), which are identified by subject number. The investigator will ensure that the CRFs are correctly completed. At the beginning of the study, a list can be generated, specifying which data fields in the Case Report Form will be used by the medical personnel for direct entry during the performance of the study. This means that these data fields in the CRF are source documents. For the other CRF data fields, the source data will be found in separate paper documentation (such as subjects' files, worksheets, etc.), *i.e.*, for these CRF data fields the separate documents are the source documents.

The completed original CRFs are the sole property of the Sponsor and should not be made available in any form to third parties without written permission from the Sponsor, except for authorized Sponsor's representatives or appropriate regulatory authorities.

The investigator or person designated by him /her will record patient data in CRFs as accurately as possible. CRF entries must be made with black ballpoint pen, pencils must not be used. The Investigator will ensure that all data are entered promptly, legibly, completely, accurately and conform to source documents, in accordance with specific instructions accompanying the CRFs, designed specifically for this study.
Correction fluid must not be used but corrections will be made by crossing the errors with a single stroke and writing the correct information next to it. All corrections must be initialled and dated. Only persons authorized by the investigator to make original CRF entries are allowed to make corrections.

After the CRF pages have been monitored (see section 12.1.2), the monitor will collect the original and first copy of each CRF page.

The original, top copy of monitored, corrected and signed CRFs for a particular patient's visit will be collected and sent by the monitor to HARRISON Data Management by a reliable courier after each monitoring visit. Only a complete set of CRF pages for those particular patient's visits/periods will be collected after verification by the CRO monitor. CRFs will be accompanied by a CRF transmittal form which will be signed by both the monitor and the person receiving the CRFs in HARRISON Data Management.

The first copy of the CRF page will be archived by the CRO and will serve as a referent tool in case of further queries or clarifications needed from Data Management.

In Data Management, brief-checking of the incoming batches of CRF pages will be performed: all the CRF pages listed on the data transmittal form must be included in the shipment and all data in the headers and footers must be consistent. Received CRFs will be logged into a CRF/Queries Tracking database (2 databases) with unique information to identify the CRF page or Query. At least the following information will be recorded:

- Date CRF page/ Query received
- Patient identification

The CRF transmittal form will be signed, dated and a copy will be sent back to the monitor in order to confirm the shipment. Valid CRFs will be moved to the data entry shelf. After being registered the CRFs will be kept in orderly way.

Data Management may generate additional requests to which the Investigator must respond by confirming or modifying the data questioned. The requests with their responses will be appended to the CRFs held by the investigator and the CRO.

12.1.2 Quality control

The study will be supervised by a monitor from Harrison Clinical Research. The study monitor will contact the investigator regularly to discuss the progress of the study and to check the study documents including the informed consent forms for completeness and consistency.

The study monitor will cross-check the data entered in the CRFs with the source data at the study site and observe the study procedures in order to verify adherence to the study protocol.

The CRFs will be checked for completeness and correctness by the monitor and data management department of HCR according to the HCR SOPs and any queries will be resolved by the investigator.

12.1.3 Data management plan and database design

Detailed information on data management is available in the data management plan specifically written for this study. The study database, data entry screens design and edit checks are defined according to the corresponding CRF(s) and the study protocol. A Data Validation Document (DVD) will be edited by the Data management and will detail all the controls and checks.

12.1.4 Data entry and validation

Prior to any data entry, data entry conventions will be written if needed. Data Capture is conducted via a data entry screen, by 2 clerks working independently. Data entry clerks will be trained for the study prior to the first data entry. All CRF's items are double entered.

Data entry will be conducted as the CRFs will go along. A comparison will be performed for each batch of the two entries by an automatic system included in the data entry software. All the discrepancies edited will be arbitrated by the data manager. Beyond this step, an automatic audit trail will track and record any modification in the file issued from the comparison. Data management will be performed according to HCR SOPs.

12.1.5 Handling of external data

External data consists in data that is not recorded on CRFs (like the laboratory data). Data may be received in electronic format or paper printout. Key variables are defined in order to uniquely identify each sample record. File and data formats are agreed with the external data provider. Any data transferred to the Data management must contain origin, date created, date sent and number of records at minimum.

12.1.6 Medical encoding

Adverse events and medical history verbatim terms are encoded using Medical Dictionary for Regulatory Activities (MedDRA) latest version available.

Concomitant medications verbatim terms are encoded using WHO Drug Dictionary (WHO DD) most recent version held by Harrison.

The database items to be encoded, the dictionaries used and their versions are specified in the data management specifications document.

12.1.7 Database lock

All data entry, verification, medical encoding and data validation activities will be finalized before the database lock. Quality Control (QC) activities will be completed to acceptable error rates. All unnecessary user privileges to the study will be removed, except for the Clinical Data Manager who will perform the database lock.

In exceptional circumstances, when critical reasons justify, there may be a need to perform updates to the database after it has been locked. A database that is locked and released for analysis will only be unlocked following Sponsor's approval if an error is identified that will significantly affect the statistical outcome of the analysis of the safety (primary and secondary) parameters or change the safety profile of the study.

12.1.8 SAE/ AE Data Reconciliation

SAEs have to be reconciled before the data review meeting and before the database freezing.

12.2 Statistical methods and determination of sample size

12.2.1 General considerations

The primary efficacy objective is to assess the minimum effective dose after three subcutaneous injections of different dose levels of BM32 as compared to placebo. The safety objective is to evaluate the relative safety and tolerability of different dose levels of BM32 compared to placebo. Immunogenicity data shall deliver supportive information about safety and the optimal dose of the trial medication.

All data obtained in this study and documented in the Case Report Forms will be listed and summarized with statistics or frequency tables as appropriate. Raw data listings and summary tables will be generated using the software SAS© Version 9 or higher.

After completion of the Case Report Form design, a Statistical Analysis Plan (SAP) will be finalized that details the planned statistical analysis in detail and may include necessary adaptations to the planned statistical analysis before unblinding of the data.

12.2.2 Determination of sample size

Three verum dose groups (i=1,2,3) with different doses of BM32 will be applied:

1) Dose D_1: 0.025mg of each BM32 component/mL (Verum Group 1)
2) Dose D_2: 0.05mg of each BM32 component/mL (Verum group 2)
3) Dose D_3: 0.1mg of each BM32 component/mL (Verum Group 3)

For comparison of the three verum doses to placebo the following primary efficacy endpoint will be used: Difference in the TNSS Score between Screening (pre treatment) and Visit 8 (post treatment).

This endpoint is assumed to follow a normal distribution (for the normal distribution assumption refer to Horn, Vollandt (1998)[5])

Let $D_1 < D_2 < D_3$ denote the three doses of the drug. Each of the 3 independent dose groups shall have the size $n=n_1=n_2=n_3$. The untreated group has the size $n_0=r*n$ with letting r=1 (1:1:1:1 group allocation). The three dose groups will be each compared to Placebo. A monotone dose-relationship is assumed, which means that the effect by drug (i.e. the difference to the control), is increasing (or at least non-decreasing) with increasing doses.

The minimum effective dose will be the drug dose, with the lowest dose to be still effective. This is the lowest dose for which the response is significantly higher than that at the zero dose level.

Following Tamhane, Hochberg, Dunnett (1996)[6] there is the need to perform **three separate one-sided α-level tests** for the individual comparisons of the different dose groups against the control group (placebo) in a stepwise manner.

1) Step 1: Starting with the highest dose D_3, this dose is compared to Placebo. If its response is not significantly higher than that of Placebo, D_3 and all other doses are considered as non-effective. The procedure stops.
   If we get significance, D_3 is considered as effective and it is proceeded to step 2.

2) Step 2: The group treated with D_2 will be compared to Placebo. If its response is not significantly higher than that of Placebo, D_2 and D_1 are considered as non-effective and D_3 is declared to be the MED. The procedure stops.
   If we get significance, D_2 is considered as effective, too, and it is proceeded to step 3.

3) Step 3: The group treated with D_1 will be compared to Placebo. If its response is not significantly higher than that of Placebo, D_1 is considered as non-effective and D_2 is declared to be the MED. The

[5] Horn, M., Vollandt, R. (1998): Sample sizes for comparisons of k treatments with a control based on different definitions on power. Biometrical Journal 40, 589-612.
[6] Tamhane, A.C., Hochberg, Y., Dunnett, C.W. (1996): Multiple test procedures for dose finding. Biometrics 52, 21-37.

procedure stops.
If we get significance, D_1 is considered as effective and declared as the MED.

All responses X_0, X_1, X_2, X_3 are assumed to have a normal distribution with the same (known) variance σ^2. The following three hypotheses are tested:

Null Hypothesis:	H_{0i}:	$\mu_i \leq \mu_0$ (i=1,2,3)
Alternative Hypothesis:	H_{Ai}:	$\mu_i > \mu_0$

Δ is the bound such that a difference $\mu_i - \mu_0 \geq \Delta$ is regarded as clinically important.

The multiple testing is done by a multiple t-testing and controls the comparison wise type I error rate.

The sample size can be estimated by the following assumptions:

Table 12-1: Results of sample size estimation

Power	90%
Significance level	5%
Test assumption	one sided, superiority testing, normal distribution
Delta Δ: Difference between Placebo and the high dose group D_3 in the TNSS Score between Screening and Visit 8 (Placebo group TNSS reduces 15% and D_3 reduces 50%)	2,65
Standard Deviation	2
The sample size formula (n per group)	$n=\lambda^2\sigma^2/\Delta^2$ with $\lambda^2=(1+1/r)\,(u_{1-\alpha} + u_{k,1/(1+r),1-\beta})^2$ [7]
Estimated number of patients per group	13
Estimated number of patients incl. drop-out rate (15%)	15
Total patient number	60

Having four treatment groups with equal patient sizes leads to a total of **60** patients.

12.2.3 Assignment of subjects to subsets

For statistical analysis, all randomized subjects will be assigned to datasets, as in Table 12-2.

Table 12-2: Analysis sets

Analysis set	Analysis set includes:
Safety analysis (SA)	All subjects who were randomized and received at least one dose of the trial medication (verum or placebo).
Full analysis (FA)	All subjects of the SA set with at least one measurement of the primary efficacy variable TNSS.
Per Protocol (PP)	All subjects of the FA set for whom no relevant protocol deviations were documented.

The decision whether a protocol deviation is relevant or not for the exclusion of subjects from the PP set will be made case by case in a data review meeting.

The primary population subset will be the FA set. All confirmatory testing is based on this subgroup. For further descriptive purposes, the same statistical procedures will be applied to the PP set.

Analysis of safety will be based on the SA dataset.

[7] Horn, M., Vollandt, R. (2000): A manual for determination of sample sizes for multiple comparisons. Institut für medizinische Statistik, Information und Dokumentation. Friedrich-Schiller Universität Jena, p.29

12.2.4 Baseline and center comparisons

Analysis of variance (ANOVA) for the main baseline characteristics will be performed. The ANOVA includes treatment effects, and will be performed to detect any global treatment effect. For categorical or binomial baseline characteristics, a Cochran-Mantel-Haenszel test will be performed, to detect any treatment imbalances.

12.2.5 Background and demographic characteristics

Demographic and background information will be summarized and displayed using descriptive statistical techniques. For categorical variables frequency tables will be presented, for continuous variables descriptive statistics such as mean, median, standard deviation, minimum, maximum and number of subjects, will be tabulated.

12.2.6 Efficacy Data

Primary efficacy endpoint

The primary efficacy endpoint is the difference in the TNSS Score between Screening and Visit 8.

Analysis of the primary efficacy endpoint

The three dose groups $D_1 < D_2 < D_3$ will be each compared to Placebo. The minimum effective dose (MED) will be the drug dose, with the lowest dose to be still effective. This is the lowest dose for which the response is significantly higher than that at the zero dose level.

Following Tamhane, Hochberg, Dunnett (1996)[8] there is the need to perform **three separate one-sided α-level tests (α=0.05)** for the individual comparisons of the different dose groups against the control group (placebo) in a stepwise manner (see section 12.2.2).

The multiple testing is done by a multiple t-testing. As indicated in section 12.2.2 (Determination of sample size), it can be assumed that asymptotically, the categorical outcomes are normal distributed. Nevertheless, this will be tested and if there are evident deviations from normal distribution, non-parametric methods will be applied (Wilcoxon-Mann-Whitney testing).

Secondary efficacy endpoints and analysis thereof:

- Difference in the total Non-Nasal Symptom Score TNNSS (TOSS and OSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the Global Symptom Score (TNSS and TNNSS) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the nasal airflow resistance (NAR) (measured using active anterior rhinomanometry) between the 6h spent in the VCC at screening and the 0-6h period spent in the VCC 4 weeks after the last injection of the treatment (Visit 8).
- Difference in the sum of wheal areas before (screening) and after the treatment (Visit 7) by dose titration of the grass pollen extract (SPT) and
- the Change in the threshold concentration of grass pollen extract necessary to provoke a positive skin reaction (SPT) between these two Visits

[8] Tamhane, A.C., Hochberg, Y., Dunnett, C.W. (1996): Multiple test procedures for dose finding. Biometrics 52, 21-37.

- Difference in the FEV1 and FEV1/FVC between Screening and Visit 8

These parameters will be analysed with t-tests in case of normality and continuous variables. In case of non-normality non-parametric methods (Wilcoxon two sample test, significance level 5%) will be applied to evaluate differences between treatment groups. For evaluation of within group differences between visits paired t-tests or Wilcoxon signed rank tests will be applied depending on the distribution and scale. Analyses of differences between visits within treatment groups will also applied for the TNSS parameter.

12.2.7 Safety Data

All safety data obtained in this study will be tabulated with descriptive group statistics (mean, standard deviation, minimum, maximum, number of valid cases).

All adverse events documented will be tabulated by system organ class and preferred term after medical coding using the Medical Dictionary for Regulatory Activities (MedDRA).

An overview of AEs will be prepared showing the number of subjects with any AEs, treatment-related AEs, serious AEs, treatment-related serious AEs and AEs leading to death/ withdrawal. AEs that are reported as "possibly", "probably/likely" or "certain" related to study medication will be considered treatment-related; missing classifications concerning study drug relationship will also be considered treatment-related.

Frequency tables of AEs concerning occurrence, seriousness, intensity and drug-relationship will be presented evaluating differences between treatment groups via Chi-Square-Test.

Frequency of local reactions (injection site reactions) and systemic reactions classified by a grading scheme according to the Position Paper of the German Society for Allergology and Clinical Immunology[9] will be compared between treatment groups with Chi-Square-Tests.

The grading is done by classification of adverse events in the following categories:

Grade 0	Local reactions like local swelling (< 15 cm and > 15 cm), redness at injection site
Grade 1	Light general reactions: e.g. mucous membrane reactions (e.g. conjunctivitis, rhinitis), general urticaria, itching, erythema, pruritus, general symptoms like cough, wheezing, headache, agitation
Grade 2	Distinct general reactions: circulatory dysregulation (change in blood pressure and pulse), dyspnoea, beginning bronchospasm, gastrointestinal reactions, anxiety
Grade 3	Strong general reactions: shock (e.g. severe hypotension, paleness), bronchospasm with severe dyspnoea, loss of consciousness, faecal and urinary incontinence
Grade 4	Overall organ failure, apnoea, circulatory arrest

The use of rescue medication will be recorded throughout the vaccination period: Intake (total amount of rescue medication needed) will be evaluated by means of comparing treatment groups with Wilcoxon-two-sample test.

Safety in terms of ECG, physical examination findings and vital signs the results obtained at the final visit will be compared with the results at baseline via descriptive measures, e.g. mean changes between baseline and final visit for vital sign data will be summarized.

[9] Kleine-Tebbe, J. , Fuchs, T. , Klimek, L., et al.: Die spezifische Immuntherapie (Hyposensibilisierung) mit Allergenen. Positionspapier der deutschen Gesellschaft für Allergologie und klinische Immunologie inhaltlich abgestimmt mit dem Ärzteverband Deutsche Allergologen. Pneumologie 2001; 55, pp. 438-44.

Shift tables for laboratory will be presented, utilising the reference ranges. These tables will summarise the change from baseline status observed for a given variable. Subjects with notable abnormal values will be identified and listed separately along with their values

Laboratory data will be summarized by presenting shift tables using normal ranges and summary statistics of raw data and change from baseline values (means, medians, standard deviations, and ranges), and by the flagging of notable values in data listings.

Concomitant medications (medications present while on study medication) will be recorded throughout the study and at premature discontinuation. These medications will be coded using appropriate standard dictionaries. The number of patients using prior or concomitant medications will be categorized by drug category and preferred term and presented via frequency tabulation.

12.2.8 Immunogenicity Data

Immunogenicity data as recorded by

- Allergen specific total IgG levels after 3 s.c. injection with BM32, as measured at screening and at Visit 7
- Allergen specific IgE levels after 3 s.c. injection with BM32, as measured at screening at screening and at Visit 7

will be analysed via graphical profiles showing the time-dependent development of the antibodies. Comparisons between treatment groups will be done via non-parametric methods (Wilcoxon-two-sample test) by using the difference of immunogenicity data between visits; or the Wilcoxon-signed rank test is performed if comparing between the visits within treatment groups.

Immunogenicity data will also deliver information concerning determination of the optimal dose.

12.2.9 Subgroup analysis

Subgroup analysis is not planned. However should there be evidence that the study population would be non-homogeneous (e.g., due to the entry factor allergy profile moderate/ severe) and that the latter may possibly influence the statistical analysis, descriptive, explorative subgroup analyses will be performed.

12.2.10 Withdrawals, drop-outs, missing values

In case of drop-outs, for the main efficacy parameter, the latest available measurement will be written forward for subsequent (missing) measurements ("last-observation-carried-forward", LOCF). Empty data fields in the CRF will generally be treated as missing values.

13 End of Trial

The end of the trial is defined as database lock.

14 List of Appendices

Appendix 1 **Time and event schedule**
Appendix 2 **ECG sample report**
Appendix 3 **Clinical Laboratory Safety Assessments**
Appendix 4 **Subjective Scoring Form**
Appendix 5 **Workbook**
Appendix 6 **Concomitant Medication Sheet and AE Sheet**
Appendix 7 **Label of Study Medication**

APPENDIX 1: Time and Events Schedule:

	Screening/Inclusion			Medication Phase			Evaluation		Follow Up
	Visit 1 Pre-Screening	Visit 2 SPT &Immunology	Visit 3 VCC Baseline	Visit 4 Injection 1	Visit 5 Injection 2	Visit 6 Injection 3	Visit 7 SPT & Immunology	Visit 8 VCC	Visit 9 Follow up
Informed Consent	✓								
Demographic Data	✓								
Medical Histiry	✓								
Concomitant Medication Check	✓	✓	✓	✓	✓	✓	✓	✓	✓
Physical Examination (full at Visit 1 & 9, brief at visits 4,5,6 & 7)	✓			✓	✓	✓	✓		✓
Vital Signs (BP, PR, BF, T)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Injection (Study drug administration)				✓	✓	✓			
ECG[1]	✓			✓	✓	✓		✓	✓
Safety Lab[2]	✓			✓	✓	✓			✓
Titrated SPT		✓					✓		
VCC Challenge[3] (incl. Subjective Scoring TNSS & TNNSS)			✓					✓	
NAR[6]			✓					✓	
Pulmonary function test (FEV1 & FVC)	✓		✓	✓	✓	✓		✓	✓
AEs /SMEs	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pregnancy Test[7]	✓			✓*	✓*	✓*	✓*	✓*	✓
Immunological parameters		✓					✓		

1 ECG, pre- and post injection, on provocation day after the provocation session
2 Laboratory Safety = haematology, biochemistry, urinalysis (done by dipstick), Serology analysed only at Screening, on provocation day prechallenge, possibility of retest at Follow Up visit
3 6hrs allergen challenge provocation session in VCC
4 TNSS = Total Nasal Symptom Score (Sum of nasal obstruction, rhinorrhoea, itchy nose and sneezing)
5 TNNSS= Total Ocular Symptom Score (Itchy eyes, Watery eyes, Red eyes) & other symptoms OSS(Cough, Itchy ears, Itchy throat)
6 NAR = Nasal Airflow Resistance, measured by active anterior rhinomanometry (assessment of obstruction)
7 Pregnancy test done by dipstick (Clearblue™) to be done at least every 28 days (*)

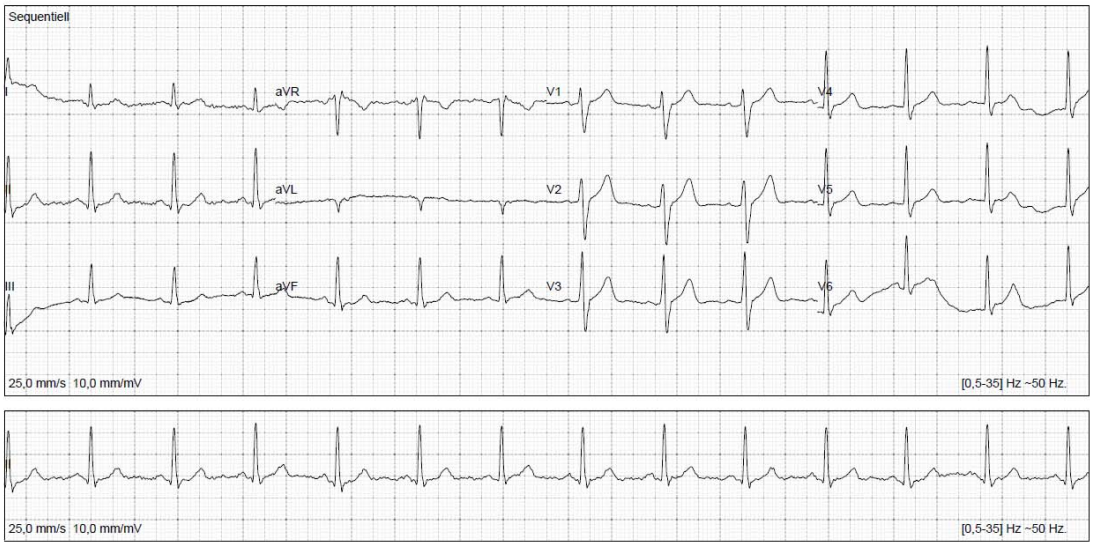
Time and Events Schedule for Challenge Chamber Sessions (Visit 3 and Visit 8)

	Pre-challenge (Baseline assessments)	Start provocation 00:00	00:15	00:30	00:45	01:00	01:15	01:30	01:45	02:00	02:15	02:30	02:45	03:00	03:15	03:30	03:45	04:00	04:15	04:30	04:45	05:00	05:15	05:30	05:45	06:00	post-challenge
Concomitant Medication Check	✓																										
Vital Signs	✓																										✓
TNSS[1]	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Challenge[5]		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Other Symptoms[2]	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
NAR[3]	✓			✓		✓		✓		✓		✓		✓		✓		✓		✓		✓		✓		✓	
Pulmonary function test	✓					✓				✓				✓				✓				✓				✓	
AEs	✓																										✓
Pregnancy test[4]	✓																										

APPENDIX 2: ECG sample report

VCC Vienna Challenge Chamber - 3007 - CS-BM32-002 - Screening

Name:	M H	Aufgezeichnet:	07.07.2011 09:02:14	Bestätigte Interpretation editiert am 08.07.2011 16:53:30 von rs
Nummer:	3007_001	Aufgenommen von:	mg	Sinusrhythmus
Geschlecht:	Männlich	Zuständiger Arzt:		
Geburtstag:	24.02.1963 46 Jahre	Arbeitsplatz:		normales EKG
		Kommentar:		
P / PQ:	115 ms / 143 ms			
QRS:	110 ms			
QT/QTc/QTd:	372 ms / 409 ms / -			
P/QRS/T Achse:	63° / 77° / 54°			
Herzfrequenz:	80 bpm			



APPENDIX 3: Clinical Laboratory Safety Assessments

Haematology

Blood will be collected according to local procedures for analysis of the following:

- White blood cell count (WBC)
- Erythrocytes
- Haemoglobin (Hb)
- Haematocrit (HCT)
- Mean cell volume (MCV)
- Mean cell haemoglobin (MCH)
- Mean cell haemoglobin concentration (MCHC)
- Platelet count
- Neutrophil count
- Lymphocyte count
- Monocyte count
- Eosinophil count
- Basophil count

Clinical Chemistry

Blood will be collected according to local procedures for analysis of the following:

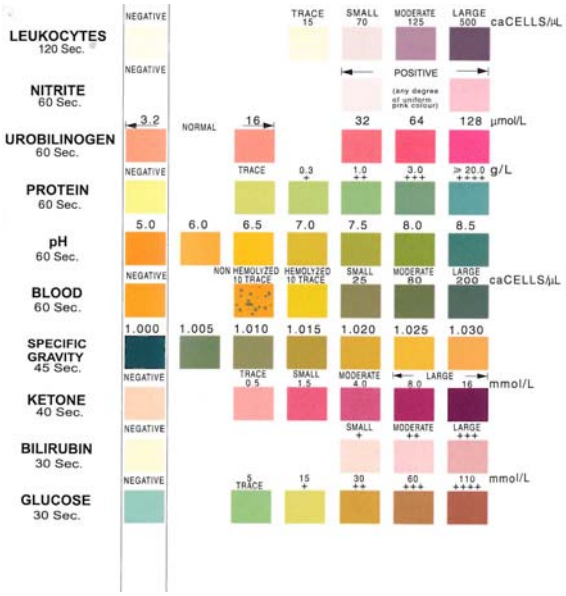
- Alkaline phosphatase
- Alanine transaminase (ALT)
- Aspartate transaminase (AST)
- Gamma-glutamyl transpeptidase (gamma-GT)
- Total bilirubin
- Creatinine
- Sodium
- Potassium
- Calcium
- Urea

Urinalysis

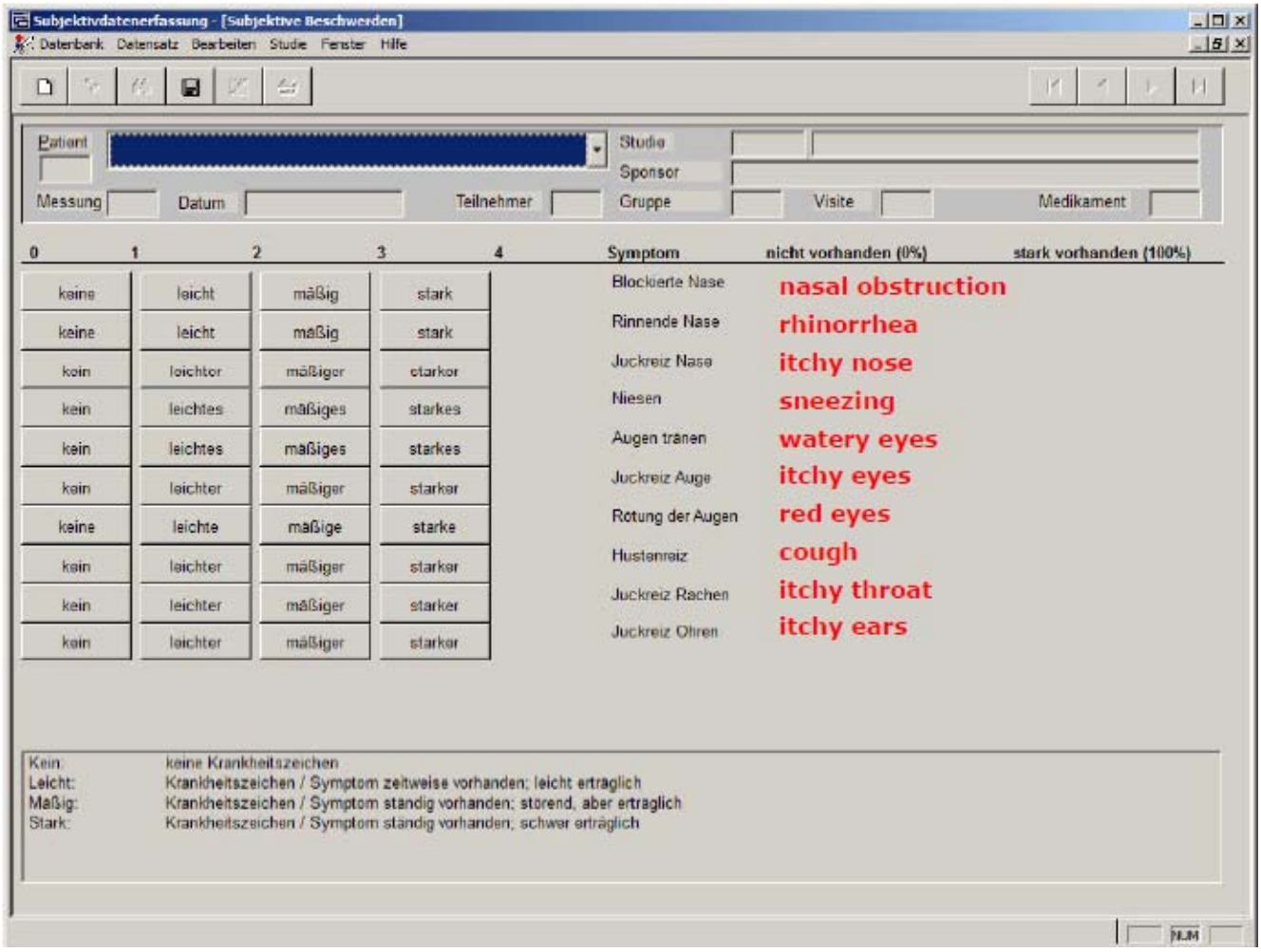
Approximately 10-20ml mid-stream urine will be collected into a container and will be tested by dipstick (Accu-Tell®).

The results are documented according to the colour template on the following Source data form:

Urine dipstick result form



APPENDIX 4: Subjective Scoring form



APPENDIX 5: Workbook

SCREENING WORKBOOK
BM32 VS. PLACEBO

Study 3007
Protocol No. **CS-BM32-002**

Datum: / / **Screening Nr.:** _____

Vorname: **Nachname:**

Geburtsdatum: **Geschlecht:** ☐ männlich ☐ weiblich

Ethnische Gruppe: ☐ kauk. ☐ asiat. ☐ afrikan. ☐ orientalisch ☐ sonstige

Einverständniserklärung:

unterzeichnet am: / / Kopie mitgegeben: ☐ Ja

sämtliche offenen Fragen von Seite des Probanden beantwortet: ☐ Ja

Begleitmedikation (innerhalb der letzten 2 Wochen):

Präparat	Dos.(mg)	Frequenz	Beginn	Ende	Cont.	Grund

Andere Krankheiten/Operationen:

Erkrankung/OP	Beginn	Ende	Therapie	1 = abgeheilt 2 = stabil 3 = instabil (bitte Kommentar!)

Allergieanamnese:

Saisonale allergische Rhinitis bei Gräserpollenbelastung (seit >1 Jahr): ☐ ja ☐ nein

Gräserpollen:

Letzter Prick-Test (≥ 3 mm): Durchmesser: _____ mm vom: _____

Letzter IgE-Befund (g6: RAST ≥ 2): Klasse: _____ vom: _____

Physiologie:

Größe: _____ [cm] Gewicht: _____ [kg] T: _____ °C

Blutdruck: / [mmHg] Puls: _____ [min^{-1}] AF: _____ [min^{-1}]

Physikalische Krankenuntersuchung:

Auffälligkeiten bei physikalischer Untersuchung: ☐ Nein ☐ Ja:_____

Asthma bronchiale: ☐ Nein wenn ja → GINA-Klassifikation:

I ☐ II ☐ III ☐ IV ☐

Inklusionskriterien:

1	Proband ist gesund	Ja	Nein
2	Proband ist zwischen 18 und 60 Jahren (inklusive) und weist eine allergische Vergangenheit auf	Ja	Nein
3	Normales EKG (12 Ableitungen, keine klinisch signifikanten Auffälligkeiten)	Ja	Nein
4	Erreichen eines TNSS von ≥6 während der ersten 2 Stunden eines 6-stündigen Aufenthalts in der Provokationskammer	Ja	Nein
5	Positiver Gräser-Prick-Test (≥3mm); nicht älter als 12 Monate	Ja	Nein
6	Positive grasspezifische IgE-Befunde (RAST Klasse ≥2 für g6 und rPhl p 1 + rPhl p 5); nicht älter als 12 Monate	Ja	Nein
7	Proband ist in der Lage zumindest 6 Stunden in der Provokationskammer zu verbringen	Ja	Nein
8	Proband ist in der Lage sein Einverständnis zur Teilnahme an der Studie zu geben und erklärt sich bereit, die Studienrichtlinien zu befolgen	Ja	Nein
9	Proband kann alle Studientermine einhalten und alle erforderlichen Messungen durchführen	Ja	Nein

Exklusionskriterien:

1	Die Probandin ist schwanger, stillt oder ist nicht bereit eine geeignete Verhütungsmethode anzuwenden.	NA	Ja	Nein
2	Veränderungen der Nase (z.B. Nasenscheidewandkrümmung, Nasenseptumperforation, Polypen etc.), Neigung zu Nasenbluten, kürzliche Nasenoperation, oder andauernde Atemwegsinfektion		Ja	Nein
3	Atemwegserkrankung außer leichtem Asthma, sofern diese keine Behandlung erfordert und mit einer normalen Lungenfunktion einhergeht		Ja	Nein
4	Proband hat innerhalb der letzten 30 Tage an einer klinischen Studie teilgenommen oder nimmt gerade an einer anderen Studie teil		Ja	Nein
5	Hyposensibilisierung (Spezifische Immuntherapie) gegenüber Gräserpollen innerhalb der letzten 2 Jahre		Ja	Nein
6	Vergangene oder aktuelle Erkrankung, die laut Prüfarzt den Studienausgang beeinflussen kann		Ja	Nein
7	Autoimmunerkrankung, Immundefekte, Immunsuppression oder Immunkomplex-induzierte Immunopathien		Ja	Nein
8	Proband reagiert allergisch auf Inhaltsstoffe der Studienmedikation		Ja	Nein
9	Einnahme verbotener Medikamente: Depot-Corticosteroide/12 Wochen, orale Steroide/8 Wochen; inhalierte Steroide/4 Wochen, Antihistaminika/4 Tage		Ja	Nein
10	Proband ist in den Augen des Prüfarztes nicht für die Teilnahme an dieser Studie geeignet		Ja	Nein
11	Allergische Symptome zum Zeitpunkt des Screening		Ja	Nein
12	Jeglicher andere Grund, der in den Augen des Investigators den Probanden als unbrauchbar für die Studie erscheinen läßt		Ja	Nein

Dokument für Begleitmedikation & besondere Vorkommnisse:

Dokument wurde ausgehändigt und erklärt: ☐ Ja

Informationen über die Anwendung der Studienmedikation:

Der Proband weiß, wie die Studienmedikation angewendet wird und welche Begleitmedikamente erlaubt beziehungsweise nicht erlaubt sind.

☐ Ja

Einhaltung der „Washout- Zeiten":

Die vorgeschriebenen Auswaschzeiten, für sämtliche im Protokoll angeführten und klinisch relevanten Medikamente, wurden erklärt und können von dem Probanden eingehalten werden:

☐ Ja

Ich wurde über den Ablauf der klinischen Studie, sowie über die darin vorkommenden Untersuchungen als auch über die dabei zu absolvierenden Pollenprovokationssitzungen aufgeklärt. Weiters habe ich die vereinbarten Termine erhalten und die an mich gestellten Anforderungen verstanden.

Name des Probanden:

Datum: Unterschrift:

Bearbeitung durch:

Datum: Unterschrift:

Kontrolle durch:

Datum: Unterschrift:

Kontaktadresse:

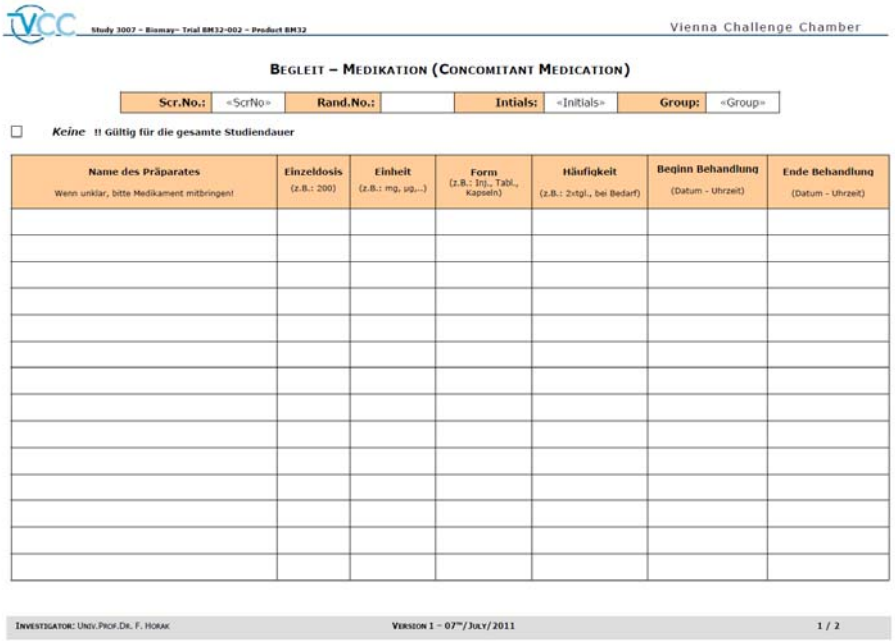
Dr. Angela Neubauer
Clinical Development

Biomay AG
Vienna Competence Center
Lazarettgasse 19/1
1090 Wien
Austria

APPENDIX 6: Concomitant Medication Sheet and Adverse Event Sheet

Concomitant medication and Adverse Event report sheet for the patients during the clinical trial:

Concomitant Medication:



Study 3007 – Biomay– Trial BM32-002 – Product BM32 Vienna Challenge Chamber

BEGLEIT – MEDIKATION (CONCOMITANT MEDICATION)

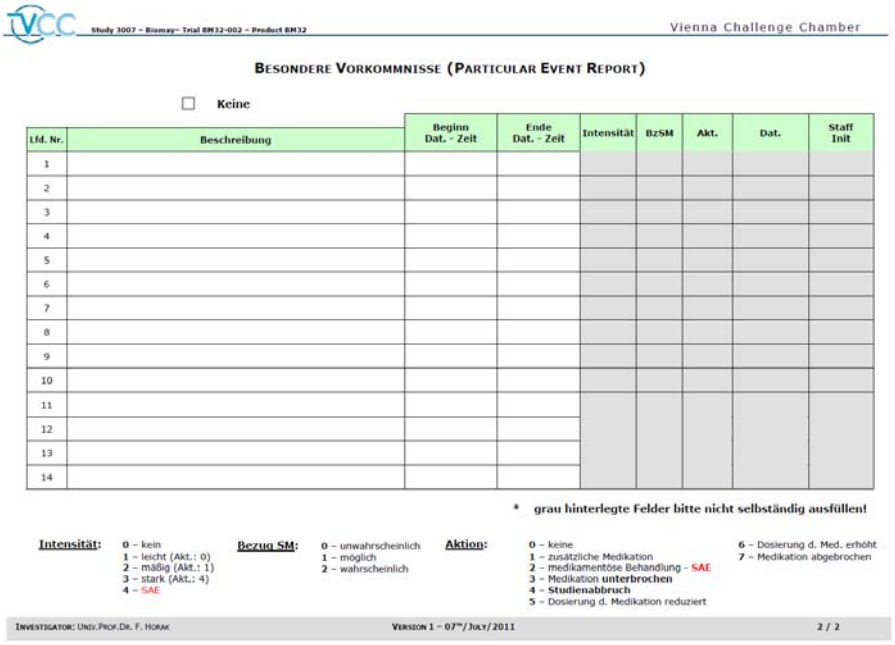
Scr.No.:	«ScrNo»	Rand.No.:		Intials:	«Initials»	Group:	«Group»

☐ *Keine* !! Gültig für die gesamte Studiendauer

Name des Präparates (Wenn unklar, bitte Medikament mitbringen!)	Einzeldosis (z.B.: 200)	Einheit (z.B.: mg, µg,...)	Form (z.B.: Inj., Tabl., Kapseln)	Häufigkeit (z.B.: 2xtgl., bei Bedarf)	Beginn Behandlung (Datum - Uhrzeit)	Ende Behandlung (Datum - Uhrzeit)

INVESTIGATOR: UNIV.PROF.DR. F. HORAK VERSION 1 – 07TH/JULY/2011 1 / 2

Adverse Event:



Study 3007 – Biomay– Trial BM32-002 – Product BM32 Vienna Challenge Chamber

BESONDERE VORKOMMNISSE (PARTICULAR EVENT REPORT)

☐ Keine

Lfd. Nr.	Beschreibung	Beginn Dat. - Zeit	Ende Dat. - Zeit	Intensität	BzSM	Akt.	Dat.	Staff Init
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								

* grau hinterlegte Felder bitte nicht selbständig ausfüllen!

Intensität: 0 – kein; 1 – leicht (Akt.: 0); 2 – mäßig (Akt.: 1); 3 – stark (Akt.: 4); 4 – SAE

Bezug SM: 0 – unwahrscheinlich; 1 – möglich; 2 – wahrscheinlich

Aktion: 0 – keine; 1 – zusätzliche Medikation; 2 – medikamentöse Behandlung - SAE; 3 – Medikation **unterbrochen**; 4 – **Studienabbruch**; 5 – Dosierung d. Medikation reduziert; 6 – Dosierung d. Med. erhöht; 7 – Medikation abgebrochen

INVESTIGATOR: UNIV.PROF.DR. F. HORAK VERSION 1 – 07TH/JULY/2011 2 / 2

APPENDIX 7: Label of Study Medication

The study medication labels (carton, vials) will comprise the following information.

Sample of outer box label:

NUR ZUM GEBRAUCH FÜR KLINISCHE STUDIEN BESTIMMT

3 x 550 µl BM32 Impfstoff oder Placebo

Studien- Nr.: **CS-BM32-002** **Pat.-Nr /Random-Nr**.: **XX**

Sterile Suspension von BM32 für subkutanen Gebrauch
Rekombinantes alumadsorbiertes Fusionsprotein
Gebrauchsanweisung: siehe KA-GCP-002 („Storage and handling of BM32 vaccine and placebo")
Lagerung: von +2 bis +8 °C; *darf nicht gefroren werden!*
Leere Packungen und ungebrauchte Produkte müssen dem Sponsor retourniert werden
Sponsor: Biomay AG, Lazarettgasse 19, 1090 Wien, Tel.:+43 1 79 66 296-100
Investigator: Prof. Dr. Friedrich Horak, Research Consult GmbH, Hütteldorferstrasse 44; A-1150 Wien; Austria

EudraCT No.: 2011-003368-64
Protokollnummer: CS-BM32-002
Zentrumsnummer: 3007

Ch.-B.: **BM32x-VAC-1101** Haltbarkeitsdatum: **05/2014**

Sample of vial label:

0,55 ml BM32 Impfsstoff oder Placebo Studien- Nr.: CS-BM32-002
sterile, wässrige Suspension von BM32 für subkutane Anwendung
Lagerung: bei 2° bis 8°C, *darf nicht gefroren werden!*
Sponsor: Biomay AG, Lazarettgasse 19, 1090 Wien, Tel.:++43 1 79 66 296-100
EudraCT-Nr: 2011-003368-64
Protokollnummer: CS-BM32-002 Pat.-Nr /Random-Nr.: XX
Ch.-B.: **BM32x-VAC-1101** Haltbarkeitsdatum: **05/2014**