2 SYNOPSIS

Name of Sponsor/company: Biomay AG	Individual Study Table Referring to Part of the Dossier	(For National Authority Use Only)
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Name of Active Ingredient(s): BM32 (containing BM321, BM322, BM325, and BM326	Page:	

Title of the study:

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.

Investigators:

Coordinating investigator: Univ.Prof.Dr. Friedrich Horak, Research Consult GmbH
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Study center:

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Publication:

Not yet available

Study period:	**Phase of Development:**
18 October 2011 (first subject signed informed consent form) 22 February 2012 (last subject completed the study)	II

Objectives

Primary Efficacy Objective

- To assess the minimum effective dose after three subcutaneous (s.c.) 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 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

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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 (nasal airflow resistance [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.

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(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 basophil activation and in the CD203c assay will be studied.

Methodology:

This was 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 were four study groups: 3 dose levels of BM32, and placebo.

Subjects were to undergo screening 3 - 28 days prior to the treatment. During screening they were to undergo a qualifying challenge session (6 hours) to check that they had 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 was defined as a TNSS of at least 6 within the first two hours of the session. The stratification of grass pollen allergic subjects into 2 groups was to be done according to the severity of their allergic reaction to grass pollen allergens by a combined scoring of reactivity by SPT and in the pollen chamber.

After screening, three injection visits at approximately 4-week intervals were planned. Three to four weeks after the final injection of BM32 subjects were to be subjected to SPT and they were to enter the VCC for a 6-hour provocation session.

Subjects were to undergo a follow-up visit 7-14 days after the final provocation session. The total duration of the study for each subject was planned to be between 14 and 20 weeks.

Number of subjects:

Planned: The target number of subjects for this study was 60. To allow for drop outs this study was to include up to 72 subjects.

Analyzed:

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Subjects included in the safety set and full analysis set: 70 total; 17 (BM32/S1), 18 (BM32/S2), 17 (BM32/S3), 18 (placebo)

Subjects included in the per protocol set: 68 total; 16 (BM32/S1), 18 (BM32/S2), 16 (BM32/S3), 18 (placebo)

Inclusion criteria:

A subject was eligible for inclusion in this study only if all of the following criteria applied:

- The subject is allergic but otherwise healthy. Healthy subjects were defined as individuals who were 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 was 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 ≥ 3mm) for grass pollen at or within 12 months preceding the screening visit.
- They have a positive test on specific IgE (ImmunoCAP or RAST) (class ≥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.

Exclusion criteria:

A subject was not eligible for inclusion in this study if any of the following criteria applied:

- 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,

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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.

Test product, dose and mode of administration, batch number:

Three s.c. injections of 400µL BM32/S1, BM32/S2, or BM32/S3 administered at 4-week intervals (-3 days / +7 days); each 400µL injection contained 10 µg (BM32/S1), 20 µg (BM32/S2), and 40 µg (BM32/S3) of each individual BM32 API (i.e., BM321, BM322, BM325, BM326), respectively.

Batch numbers: BM32x-VAC-1101

Duration of treatment:

Three injections at 4-week intervals

Treatment with placebo, dose and mode of administration, batch number:

Three s.c. injections of 400µL placebo (aluminum hydroxide only) administered at 4 week intervals (-3 days / +7 days);

Batch number(s): BM32x-VAC-1101

Criteria for evaluation:

Efficacy

Primary efficacy endpoint:

The primary efficacy endpoint was the difference in the TNSS (nasal obstruction, rhinorrhoea, itchy nose and sneezing) between spending 6h in the VCC at VCC baseline (Visit 3) and spending 6h in the VCC 4 weeks after the last injection of the treatment (Visit 8).

The secondary efficacy endpoints were:

- Difference in the 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 Global Symptom Score between the 6h spent in the VCC at VCC baseline visit 3 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 (measured using active anterior rhinomanometry) between the 6h spent in the VCC at VCC baseline visit 3 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 (Visit 2) and after the treatment (Visit 7) by dose titration of the grass pollen extract measuring the difference in

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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 VCC baseline Visit 3 and Visit 8

In the Statistical Analysis Plan (SAP), version 1.0, dated from 18 April 2012, the following specifications were added:

During the challenge session in the VCC only the assessments made between 2 and 6 hours were considered significant for the analysis. TNSS, TNNSS, global symptom score, and NAR were therefore analyzed by calculating the mean of all assessments made within the 2-6-hour period. The mean value is specified in the following as $TNSS_{2\text{-}6h}$, $TNNSS_{2\text{-}6h}$, global symptom score (2-6h), and $NAR_{2\text{-}6h}$.

Additionally, the area under the curve (AUC) within the 2-6-hour period ($AUC_{2\text{-}6h}$) of the TNSS, TNNSS, and global symptom score was to be calculated.

Safety and tolerability endpoints:

- Adverse events
- Rescue medication
- Vital signs, physical examination, ECG
- Clinical laboratory

Immunogenicity endpoints:

- Changes in specific IgG levels after 3 s.c. injections of BM32, as measured at screening and Visit 7
- Changes in specific IgE levels after 3 s.c. injections of BM32, as measured at screening and Visit 7
- Changes 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 in the sensitivity to recombinant grass pollen allergens and timothy grass pollen extract as determined by allergen-induced basophil activation using the CD203c assay after 3 s.c. injections of BM32, as measured in blood or serum samples collected at screening (baseline) and after the last injection of the treatment (Visit 7).

Statistical methods:

Efficacy analysis:

- **Analysis of the primary efficacy endpoint:**
  The primary efficacy endpoint (difference in the $TNSS_{2\text{-}6h}$ between Visit 3 and Visit 8) was analyzed by a stepwise procedure. The difference in the TNSS $AUC_{2\text{-}6h}$ between Visit 3 and Visit 8 was evaluated as a secondary endpoint.
  The three BM32 dose groups were each compared to placebo using one-sided α-level tests (α=0.05). The minimum effective dose (MED) was defined as the lowest dose for which the response was significantly higher than that at the zero dose level.
  Multiple testing was to be performed using a multiple t-testing procedure. If there were evident deviations from normal distribution, non-parametric methods were to be applied (Wilcoxon-Mann-Whitney testing).
  All following tests were performed at a 5% significance level with no adjustments for multiplicity. Within group differences between visits were to be evaluated with paired t-tests

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or Wilcoxon signed rank tests depending on the distribution. In addition ANCOVAs were performed with factors treatment and strata (moderate and severe allergy).
The $TNSS_{2\text{-}6h}$ and TNSS $AUC_{2\text{-}6h}$ at Visits 3 and 8 and the differences between Visit 3 and Visit 8 were analyzed with descriptive statistics and displayed graphically (mean and 95% CI) for each group. The $TNSS_{2\text{-}6h}$ and TNSS $AUC_{2\text{-}6h}$ at Visits 3 and 8 were to be compared between treatment groups by means of t-tests or Wilcoxon-Mann-Whitney tests.

- **Analysis of secondary efficacy endpoints:**
  The $TNNSS_{2\text{-}6h}$, TNNSS $AUC_{2\text{-}6h}$, global symptom score (2-6h) and $AUC_{2\text{-}6h}$ of the global symptom score were summarized with descriptive statistics by visit. Descriptive measures of the $TNNSS_{2\text{-}6h}$ and the global symptom score (2-6h) were graphically displayed with bar charts for each treatment group and visit.
  - **Difference in the TNNSS between Visit 3 and Visit 8**
    The difference between Visit 3 and Visit 8 in the $TNNSS_{2\text{-}6h}$ and TNNSS $AUC_{2\text{-}6h}$ was summarized with descriptive statistics. Comparisons between each BM32 group and placebo were to be performed with two-sided t-tests (or Wilcoxon two sample tests in case of non-normality, 5% significance level). Within group differences between visits were to be evaluated with paired two-sided t-tests or Wilcoxon signed rank tests depending on the distribution (5% significance level).
  - **Difference in the Global Symptom Score between Visit 3 and Visit 8**
    The difference in the global symptom score (2-6h) and the AUC2-6h of the global symptom score was evaluated in the same manner as the TNNSS.
  - **Difference in the NAR between Visit 3 and Visit 8**
    The percent change of the total $NAR_{2\text{-}6h}$ value (sum of the left and right value) compared to the pre-challenge value was to be compared between each BM32 group and placebo at Visit 3 and Visit 8 using two-sided t-tests (or Wilcoxon two sample tests in case of non-normality, 5% significance level).
  - **Change in skin reaction to grass pollen allergens (SPT) before (Visit 2) and after treatment (Visit 7)**
    SPT results were summarized with frequency tables. The difference in the highest positive dilution level between Visit 2 and Visit 7 was summarized with descriptive statistics. Comparisons between BM32 treatment groups and placebo were to be performed by Wilcoxon-two sample tests for each visit. In between comparisons within each treatment group were to be performed with the Wilcoxon signed rank test.
    Wheal areas and the difference in the total sum of wheal areas between Visits 2 and 7 were summarized with descriptive statistics and compared with Wilcoxon-two-sample tests.
  - **Difference in FEV1 and FEV1/FVC between VCC baseline Visit 3 and Visit 8**
    The maximum percent decrease in FEV1, FVC and FV1/FVC compared to the pre-challenge value was to be compared between each BM32 group and placebo at Visits 3 and 8 using t-tests in case of normality. In case of non-normality non-parametric methods (Wilcoxon two sample tests, significance level 5%) were to be applied.
    FEV1 (readings 1–3, mean of the three readings, predicted, % predicted of highest reading) measurements were summarized with descriptive statistics.

Safety and tolerability analysis:

Vital signs, results of the physical examination (normal/abnormal), and ECG findings (normal/abnormal) were summarized with descriptive statistics or frequency tables. For vital signs, changes to baseline were calculated for each visit and differences between pre- and

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post-injection were evaluated.

Medical history was summarized with frequency tables using MedDRA codes (system organ class [SOC] and preferred term [PT]). Previous medication, concomitant medication and rescue medication were coded using the WHO-Drug Dictionary Version Q2/ 2011. Concomitant medications were summarized with frequency tables (number and percentage of subjects, number of events) by anatomic group levels ATC 2, ATC 4 and WHO Drug Dictionary PT. Previous medications were listed only.

Number and percentage of subjects, who needed rescue medication, were tabulated by visit. Comparisons between treatment groups were to be performed for each visit using the Chi-Square test or exact Fisher's test in case of small cell frequencies.

Results of clinical hematology, clinical chemistry and urine dipstick test were evaluated with descriptive statistics; the numbers of values within and outside the normal range were counted for each visit.

Frequency tables were prepared, grouped by SOC and PT, showing all AEs, all treatment-related AEs, serious AEs, AEs by intensity and AEs that lead to study discontinuation. The same tables were prepared for *SIT-specific* AEs. SIT-specific AEs were graded according to Kleine-Tebbe et al. (2001) and summarized with frequency tables by grade, SOC and PT. Comparisons were performed using a Chi-square-test (significance level 5%).

Immunology analysis:

Allergen- and carrier- (PreS) specific antibody levels were listed. Selected parameters were analyzed with:

- Descriptive statistics
- Changes between Visit 2 and 7 were calculated and summarized with descriptive statistics.
- Comparisons between treatment groups were to be done via non-parametric methods (Wilcoxon-two-sample test) using the difference of immunogenicity data between visits. The Wilcoxon-signed rank test was to be performed to compare intra-subject changes between visits within treatment groups.
- Graphical profiles showing the time-dependent development of the antibodies were to be presented via bar charts/ line plots.

Allergy-related immunological parameters were listed.

SUMMARY - CONCLUSIONS

EFFICACY RESULTS:

The primary endpoint of the study was the difference in the Total Nasal Symptom Score between Visit 3 and Visit 8 upon exposure in the Vienna Challenge Chamber for 6 hours.

Following vaccination with BM32, the $TNSS_{(2\text{-}6h)}/AUC_{(2\text{-}6h)}$ TNSS significantly decreased with mean changes of -1.41/-341.3 (BM32/S2) and -1.34/-328.7 (BM32/S3), while the mean changes observed in the BM32/S1 group (-0.73/-174.3) and placebo group (-1.02/-240.8) were not significant.

However, this trend did not reach statistical significance if the mean change of TNSS of either BM32/S3 or BM32/S2 was compared to that of placebo.

The same trend was observed for the Total Non-Nasal Symptom Score $_{(2\text{-}6h)}/AUC_{(2\text{-}6h)}$ TNNSS with the following mean changes from baseline: -0.74/-180.9 (BM32/S1), -1.61/-394.2 (BM32/S2), -1.14/-281.0 (BM32/S3), and -0.92/-214.2 (placebo). Consequently this trend was also reflected in the global symptom score.

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There were no significant differences between groups in the mean nasal airflow resistance (2-6h) calculated as % change from pre-challenge measured at baseline and Visit 8. The changes from baseline were 10.94 (BM32/S1), -7.05 (BM32/S2), 15.23 (BM32/S3) and 8.15 (placebo). Positive values indicate an improvement.

The results of the titrated skin prick test with standardized grass pollen extract performed before (Visit 2) and after the treatment (Visit 7) showed a clear trend towards a dose-dependent decrease in skin reactivity upon treatment with BM32. For the mean total sum of wheal areas, due to fairly large data variance, this trend was not strong enough to show a significant difference between the groups. However, looking at the parameter highest dilution level that induced a skin reaction, there was a significant difference between the groups BM32/S3 and placebo, indicating a reduced skin sensitivity against grass pollen extract. There was no difference between the groups of BM32/S1 and placebo.

Because of the natural decline of grass pollen sensitivity due to absence of allergen exposure in the time between pollen seasons, all groups including the placebo group showed a significantly decreased skin reactivity when the results of the titrated skin prick test to standardized grass pollen extract from Visit 7 were compared with those of Visit 2.

No clinically relevant decreases in FEV1/FVC upon challenge were observed in the four groups. At Visit 8 the decrease in FEV1/FVC upon challenge ranged from -2.60% to -5.63% in the BM32 groups compared to -1.92% in the placebo group. Effects of vaccination with BM32 on grass pollen-induced reduction of lung function could therefore not be assessed.

SAFETY RESULTS:

All 70 subjects experienced at least one AE. A total of 313 AEs were reported (319 MedDRA PTs), of which 237 were considered related to the study treatment.

The most common treatment-related AEs were local reactions, including most frequently injection site reactions (85.7%) and injection site urticaria (30.0%) (i.e., the MedDRA preferred term for the AE "wheal at the injection site"), pain at the injection site (8.6%), and injection site pruritus (2.9%). Other treatment-related AEs that occurred in two or more subjects included allergic rhinitis (7.1%), nasal congestion (2.9%), headache (7.1%), and urticaria (4.3%). All other treatment-related AEs occurred in single subjects only.

The majority of treatment-emergent AEs were mild or moderate. Severe injection site reactions (>15cm diameter) occurred in 29.4% (BM32/S1), 61.1% (BM32/S2) and 70.6% (BM32/S3) of the subjects who received BM32 (no cases were observed in the placebo group). Other severe AEs included abscess limb (which according to the investigator was not close to the injection site and therefore – according to them - the relatedness to study drug is questionable) in one subject in the placebo group (5.6%), allergic rhinitis in one subject in the BM32/S3 group (5.9%) and urticaria in one subject in the BM32/S2 group (5.6%).

Two hundred and nine (88.2%) treatment-emergent AEs occurred with a delayed onset on the day of injection or the next day. Twenty eight (11.8%) treatment-emergent AEs occurred within 30min after the injection of IMP. The majority of those were local (injection site urticaria, i.e. a wheal at the injection site), there was one case each of the following light systemic reactions: Allergic rhinitis, wheals at former injection site, slight dizziness, flush, itchy nose, and redness/itching of palms.

SIT-specific AEs (a term used in this study for adverse events which are known to be associated with specific immunotherapy) observed in this study were of Grade 0 (local reactions) and Grade 1 (light general reactions) according to the classification of the German

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Society of Allergology and Clinical Immunology (Kleine-Tebbe et al., 2001).

The frequencies of SIT-specific AEs were not significantly different between treatment groups, except for injection site urticaria (one wheal at the injection site) (p-value: 0.008) and allergic rhinitis (p-value: 0.021). Injection site urticaria did not occur in the placebo group, but was reported in 29.4% (BM32/S3), 44,4% (BM32/S2), and 47.1% (BM32/S1) of subjects after injection of BM32 Allergic rhinitis occurred in 5.9% of subjects in the BM32/S1 group and in 23.5% of subjects in the BM32/S3 group, but not in the other two dose groups. It was delayed in all but 1 case.

No SAEs were reported. One subject was prematurely withdrawn from the study after he had received two injections of BM32/S3. Because of repeated occurrence of adverse events following IMP administration (moderate systemic reactions after injection 1 and a severe local reactions after injection 2) the subject chose to discontinue the study.

No clinically relevant trend was observed in clinical laboratory parameters and vital signs. No relevant physical abnormalities were reported and there were no abnormal ECG recordings.

No anaphylactic reaction occurred in any of the groups and accordingly, no "rescue medication" as defined per protocol was needed. However, there was a significant difference between groups in the intake of pre- or co-medication related to IMP administration (the term "rescue medication" was also used by the investigator for any medication given to the patients to treat or prevent any treatment-related adverse reaction). More subjects required medication after vaccination with BM32 (66.7% to 82.4%) compared to placebo (5.6%). Most frequently subjects received antihistamines such as desloratadine, or anti-inflammatory medications such as diclofenac and dimetindene.

IMMUNOLOGY RESULTS:

Measurements of allergen specific IgE antibodies showed different trends depending whether a test was used which measured IgE binding under conditions of allergen excess (i.e., ImmunoCAP), with small amounts of immobilized allergen (i.e., ISAC chip) or intermediate amounts of immobilized allergen (i.e., ELISA). Under conditions of allergen excess, IgE binding is not influenced by competing allergen-specific IgG and thus changes of allergen-specific IgE levels can be quantified and determined. Under conditions of low amounts of immobilized allergen (i.e., ISAC chip), allergen-specific IgG antibodies can compete with allergen-specific IgE binding and blocking effects of IgG on IgE binding are visualized.

Measurements of allergen specific IgE antibodies by quantitative ImmunoCAP measurements showed that vaccination with BM32 did not induce any relevant increases of allergen-specific IgE levels and thus had low allergenicity.

The ELISA IgE results were in agreement with the ImmunoCAP measurements because only marginal changes of IgE levels were observed in the BM32-treated groups. A decline of allergen-specific IgE levels was measured by ImmunoCAP for the placebo group reflecting the natural decline of allergen-specific IgE levels outside the grass pollen season.

The ISAC chip which measures IgE binding to low amount of solid phase bound allergens showed that IgE antibody levels against the allergens Phl p 1, Phl p 2, Phl p 5, and Phl p 6 significantly decreased in all groups, with the exception of a non-significant decrease in Phl p 2 and Phl p 6 specific antibodies in the placebo group. The decrease was generally stronger in the BM32-treated groups which can be explained by the induction of allergen-specific IgG competing with IgE for allergen binding.

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Significant increases in allergen specific IgG antibodies specific for Phl p 1, Phl p 2, Phl p 5, and Phl p 6 were clearly shown in the ELISA and the ISAC chip test for all BM32 dose groups. No significant changes in IgG antibodies were observed in the placebo group. The observed differences between groups were significant.

Increases in IgG antibodies specific for Phl p 1, Phl p 5, and Phl p 6 were also observed in the ImmunoCap test in all BM32-treated groups, but only Phl p 5- and Phl p 6-specific IgG antibodies increased significantly. No significant increase was observed in the placebo group. A significant difference between groups was only observed for Phl p 6-specific IgG antibodies.

Allergen-specific IgA and IgM antibodies and antibodies specific for PreS were only measured by ELISA.

Phl p 5- and Phl p 6-specific IgA antibodies showed a marginal but statistically significant increase in all BM32 dose groups, whereas no significant change was observed in the placebo group. The differences between groups were significant. Phl p 1- and Phl p 2-specific IgA antibodies also increased, but not significantly in all groups, and there were no significant differences between groups.

The changes from baseline in allergen specific IgM antibodies were marginal and not significantly different between groups. There were no significant changes from baseline with the exception of a marginal but statistically significant increase in Phl p 1-specific antibodies in the BM32/S3 group.

IgG antibodies specific for PreS showed a strong and significant increase after vaccination with BM32, whereas no significant change was observed in the placebo group. IgE antibodies to PreS showed a marginal but statistically significant increase in the BM32-treated groups but not in the placebo group. The changes from baseline in IgG and IgE were significantly different between groups. No significant changes were observed in PreS-specific IgM and IgA antibodies except for a significant but only marginal change in IgA observed in the BM32/S3 group.

CONCLUSION:

- Even though BM32 vaccination did not lead to a statistically significant difference in $TNSS_{2\text{-}6h}$ (VCC) between Visit 3 and Visit 8 as compared to placebo, the intra group difference reached statistical significance for the BM32/S3 and BM32/S2 groups, while this was not the case for the BM32/S1 and placebo group.
- In titrated skin sensitivity testing, a significant reduction of sensitivity was observed in a threshold analysis for BM32/S3. In a sum of wheal area analysis, dose dependent trends were observed. While in the groups BM32/S3 and BM32/S2 the reduction in the sum of wheal areas was larger than in the placebo group, BM32/S1 did not differ from placebo.
- BM32 was safe and well tolerated at all BM32 dose levels. Although overall frequencies of injection site reactions were not significantly different between different BM32 doses or the highest dose of BM32 and placebo treatments, the frequency of severe injection site reactions tended to be higher in the BM32/S2 and BM32/S3 groups than in the BM32/S1 group and there was no severe injection site reaction in the placebo group. SIT-specific systemic reactions were all mild (grade 1) and the large majority occurred with a delayed onset, either on the day of injection or the next day.
- Vaccination with BM32 induced IgG antibodies which recognized the 4 major allergens of grass pollen Phl p 1, Phl p 2, Phl p 5, and Phl p 6.
- Vaccination with BM32 did not induce significant relevant increases of allergen-specific IgE

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Name of Sponsor/company: Biomay AG	Individual Study Table Referring to Part of the Dossier	(For National Authority Use Only)
Name of Finished Product: not decided	Volume:	
Name of Active Ingredient(s): BM32 (containing BM321, BM322, BM325, and BM326	Page:	

levels as measured by ImmunoCAP.

- Taken together the data suggest the dose levels S3 and S2 are likely to have a beneficial effect on allergic symptoms following grass pollen challenge, while dose level S1 was clearly found to be non-effective. Since S3 and S2 could not be clearly differentiated, they will both be examined in a subsequent phase IIb trial.

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