STATISTICAL ANALYSIS PLAN

Final Version, dated 22-Jun 2018

Phase II Dose-finding Study of ASP4070

—A Randomized, Double-blind, Placebo-controlled, Dose-finding Study in Patients With Cedar Pollinosis Using an Environmental Exposure Chamber—

< Pollinosis Symptoms Survey Period and Additional Study Period >

ISN: 4070-CL-0020

Astellas Pharma Inc. (API)

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I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
ANCOVA	Analysis of Covariance
BAT	Basophil Activation Test
BMI	Body Mass Index
CI	Confidence Intervals
CSR	Clinical Study Report
GD	Global Development
IAS-2	Immunological Analysis Set 2
ICH	International Conference on Harmonization
JRC	Japanese Red Cedar
JRQLQ	Japanese Rhinoconjunctivitis Quality of Life Questionnaire
LAMP	Lysosomal Associated Membrane Protein
LLOQ	Lower Limit of Quantitation
PD	Pharmacodynamic
PGx	Pharmacogenomics
PK	Pharmacokinetic
PSAS	Pollinosis Symptom Analysis Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDA	Secondary Data Analysis
TLF	Tables, Listings and Figures
TNNSS	Total Non-nasal Symptom Score
TNNSMS	Total Non-nasal Symptom Medication Score
TNSMS	Total Nasal Symptom Medication Score
TNSS	Total Nasal Symptom Score
TSMS	Total Symptom Medication Score
TSS	Total Symptom Score
ULOQ	Upper Limit of Quantitation
WHO-DD	World Health Organization Drug Dictionary

List of Key Terms

Terms	Definition of terms
Baseline	Observed values/findings which are regarded observed starting point for comparison.
Endpoint	A variable that pertains to the trial objectives
Investigational period	Period of time where major interests of protocol objectives are observed, and where the test drug or comparative drug (sometimes without randomization) is usually given to a subject, and continues until the last assessment after completing administration of the test drug or comparative drug.
Randomization	The process of assigning trial subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential subjects for enrollment in a trial.
Screening period	Period of time before entering the investigational period, usually from the time of starting a subject signing consent until just before the test drug or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first site initiation date to the last site completing the study.
Variable	Any quantity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

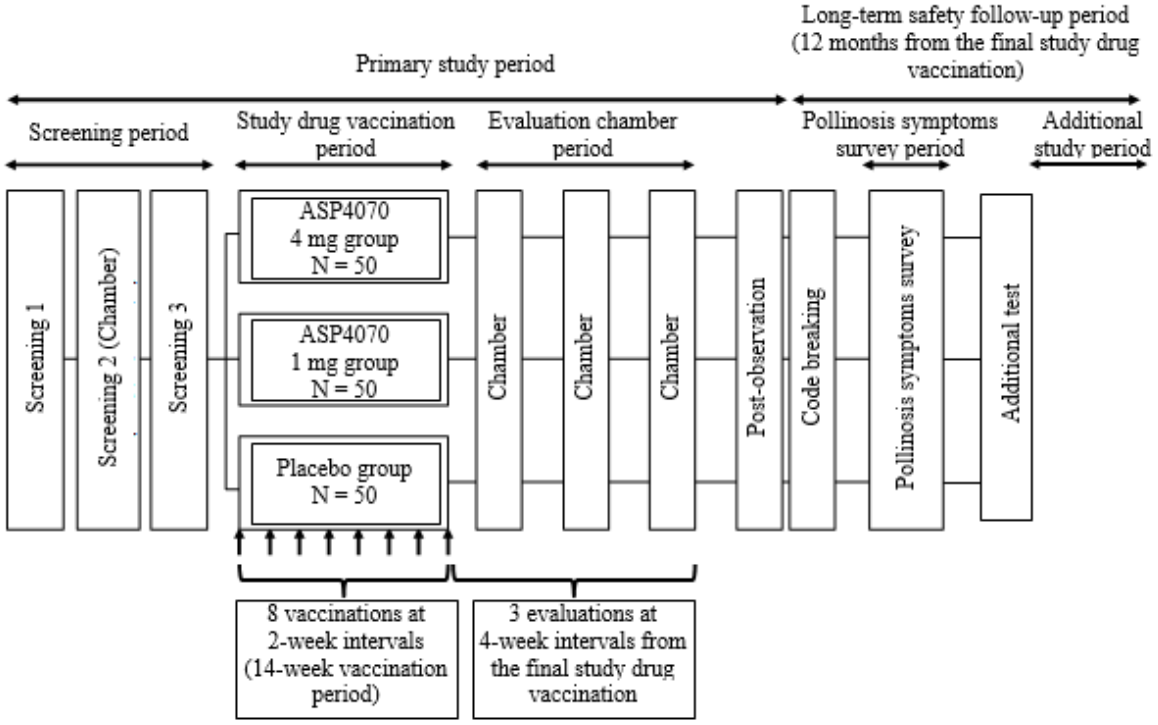
This Statistical Analysis Plan (SAP) for the pollinosis symptoms survey period and additional study period contains a more technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes detailed procedures for executing the statistical analysis of the primary and secondary endpoints and other data.

The SAP is finalized and signed prior to database hard lock at the end of the additional study period.

This statistical analysis is coordinated by the responsible biostatistician of GD, API. Any changes from the analyses planned in the SAP will be justified in the Clinical Study Report (CSR).

2 FLOW CHART AND VISIT SCHEDULE

Flow Chart



- After code breaking, the Sponsor will be unblinded, but the study will be continued with the investigator, sub-investigator, study coordinator, and subjects remaining blinded.
- Cedar pollinosis symptoms will be surveyed during the pollinosis symptoms survey period (01 Feb 2018–31 Mar 2018).
- The additional test of immune responses will be performed during the long-term safety follow-up period on subjects who have completed the pollinosis symptoms survey period and have provided additional consent to the additional test.
- Subjects will report whenever SAEs occur during the long-term safety follow-up period. Even if there is no contact from a subject, in principle, the subject will be asked about the occurrence of SAEs at 6 months and 12 months after the final vaccination. With regard to subjects who have discontinued the study during the primary study period, if the study drug had been vaccinated even once, then safety information (SAEs) will be collected for 1 year after the final vaccination.

Table 1: Schedule of Assessments

Item \ Visit day	Primary study period															After Pollinosis Symptoms Survey	Additional test
	Screening period			Study drug vaccination period								Evaluation chamber period			Post-observation	Discontinuation[3)]	
	Screening visit 1	Screening visit 2[1)]	Screening visit 3[2)]	Day 1	Day 15	Day 29	Day 43	Day 57	Day 71	Day 85	Day 99	Day 127	Day 155	Day 183			
Period (Day)			28 ± 7 from Screening visit 2[4)]	1	15 ± 3	29 ± 3	43 ± 3	57 ± 3	71 ± 3	85 ± 3	99 ± 3	127 ± 7	155 ± 7	183 ± 7	7 ± 3 from Day 183	–	Apr 2018	May 2018[21)]
Informed consent	○																○[20)]	
Inclusion/exclusion criteria	○	○	○	○														
Randomization				○														
Target disease	○	○	○															
Subject demographics	○																	
Physical examination[5)]	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Vaccination				○	○	○	○	○	○	○	○							
Chamber evaluation		○										○	○	○				
Vital signs[6),7)]	○	○	○	○	○	○	○	○	○	○	○	○	○	○		○		
12-lead electrocardiogram[6)]	○			○								○		○		○		
Immunological test[8)]	○																	
JRC allergy test[9)]	○		○															
Allergy test other than JRC[8)]	○																	
Pregnancy test (urine)[6),10)]	○			○		○		○		○		○	○	○		○		
Hematology test, blood biochemistry test, and urinalysis[6),11)]	○		○	○				○				○		○		○		
Consent for PGx[12)]				◄	―	―	―	―	―	―	―	―	―	►				
PGx blood sampling[6),13)]				◄	―	―	―	―	―	―	―	―	―	►				
Consent for SDA				○														
SDA blood sampling[6),14)]				○								○		○				
Parameters[6),15),16)]				○				○				○	○	○		○		○[22)]
Adverse events	◄	―	―	―	―	―	―	―	―	―	―	―	―	―	►	○		
Local/Systemic Reaction Survey Diary[17)]				◄	―	―	―	―	―	―	►							
Symptoms Survey After Chamber Period Diary[18)]												◄	―	►				

Pollinosis Symptoms Survey Diary[19)]																◄——►	

○: To be performed

1) Based on the results of tests and observations conducted at Screening visit 1, only those subjects who satisfy the Inclusion Criteria and do not fall under the Exclusion Criteria will be the target of Screening visit 2 onward.
2) Based on the symptom score at the chamber evaluation at Screening visit 2, only those subjects who satisfy the inclusion criterion 5 will be the target of Screening visit 3 onward.
3) Only those subjects who discontinue the study before Day 183 will be the target. In principle, discontinuation tests are conducted upon the decision to discontinue.
4) Conducted no later than 7 days prior to Day 1.
5) For visits with study drug vaccination, conduct physical examination (subjective symptoms, objective findings) 1 hour after study drug vaccination before allowing the patient to leave the clinic.
6) For visits with study drug vaccination and chamber evaluation, conduct all other tests before vaccination and chamber evaluation.
7) Refer to “5.4.3 Vital Signs” on the protocol for test items.
8) Refer to “5.2.2 Medical History” on the protocol for test items.
9) Refer to “5.2.3 Diagnosis of the Target Disease, Severity, and Duration of Disease” on the protocol for test items.
10) For female subjects only. Not required for subjects who are post-menopausal (defined as at least 1 year without any menses) at Screening visit 1 or subjects who are surgically sterile or have had hysterectomy or oophorectomy, and the possibility of pregnancy can be negated.
11) Refer to “5.4.4 Laboratory Assessments” on the protocol for test items.
12) Obtain before conducting PGx blood sampling.
13) To be conducted once during the period from Day 1 to Day 183.
14) To be conducted only if blood is sampled when sample processing is possible.
15) Refer to “5.7.1 Immune Response Assessment (Parameters)” on the protocol for test items.
16) BAT is to be conducted only if blood is sampled when sample processing is possible. If blood is not sampled on Day 1, then further blood sampling will be unnecessary.
17) To be entered every day from each day of study drug vaccination to 14 days after vaccination.
18) To be entered every day until 3 days after leaving the chamber (day of chamber evaluation, and 1, 2, and 3 days after leaving).
19) Survey will be conducted with the target as subjects who have completed the primary study period. To be entered every day from 01 Feb 2018 to 31 Mar 2018.
20) Informed consent for the additional test.
21) If a systemic steroid or an immunosuppressant (excluding external use on the skin) is used after the end of the pollinosis symptoms survey period up to informed consent for the additional test, the additional test will be performed 28 days after the last dose of the drug or later.
22) The additional test will determine BAT and specific IgE antibody (anti-JRC) only.

3 STUDY OBJECTIVE(S) AND DESIGN

3.1 Study Objective(s)

To evaluate the efficacy, safety, and dose response for ASP4070 vaccinated in patients with cedar pollinosis.

3.2 Study Design

This study is a randomized, double-blind, placebo-controlled, dose-finding study.

The efficacy, safety, and dose response when ASP4070 (mixture containing equivalent amount of Cry j 1-LAMP plasmid and Cry j 2-LAMP plasmid, 4 mg/0.4 mL or 1 mg/0.4 mL) will be intradermally vaccinated 8 times at 14-day intervals will be evaluated with placebo (physiological saline) as the control. The study will conduct cedar pollen exposure using an environmental exposure chamber (OHIO Chamber; hereinafter, "chamber"), and the efficacy and dose response will be evaluated using clinical symptoms (nasal and eye symptoms) as indicators.

Two factors, "Class from results of Japanese red cedar (JRC) pollen-specific IgE antibody test performed at Screening visit 1" and "Change in the mean of total 3 nasal symptom (sneezing, nasal discharge, and nasal congestion) score during 120 to 180 minutes after start of cedar pollen exposure in the chamber as compared to the score before cedar pollen exposure at Screening visit 2," will be used as stratification factors to randomly allocate subjects into three groups—ASP4070 4 mg group, ASP4070 1 mg group, or placebo group—in a ratio of 1:1:1, and the subjects will be vaccinated with the study drug.

After a set period after the final vaccination of the study drug, re-exposure to cedar pollen in the chamber will be conducted and the efficacy will be evaluated using clinical symptoms as indicators. Efficacy evaluation will be conducted 4, 8, and 12 weeks after the final vaccination to confirm the timing of effectiveness. The primary study period of the study will be until the end of the one week of post-observational period after the final evaluation in the chamber on Day 183 (12 weeks after the final vaccination).

The treatment code will be broken when the primary study period is completed and all data entered in case report forms are fixed. Even after code breaking, the study will be continued with the investigator, sub-investigator, study coordinator, and subjects remaining blinded.

Subjects who have completed the primary study period will be the target of a survey on cedar pollinosis symptoms during the pollinosis symptoms survey period (01 Feb 2018–31 Mar 2018). Subjects will make entries in the Pollinosis Symptoms Survey Diary every day on information regarding nasal and eye symptoms. If a subject experiences intolerable nasal and eye symptoms during this period, then the use of rescue drugs will be allowed.

An additional test of immune responses will be performed during the long-term safety follow-up period on subjects who have completed the pollinosis symptoms survey period and have provided additional consent to the additional test. The additional test will determine the

parameters (specific IgE antibody [anti-JRC] and basophil activation test [BAT]) after the end of the cedar pollen dispersal season (May 2018).

After the end of the primary study period, safety information (SAE) will be collected for approximately 9 months (1 year from the final vaccination of the study drug) as the long-term safety follow-up period. Safety information (SAE) will be collected for 1 year after the final vaccination of the study drug even from subjects who discontinue their participation in the study during the primary study period if they have received at least one vaccination of the study drug.

Among subjects in the placebo group or optimal dose group (ASP4070 4 mg group or 1 mg group) who complete the primary study period, those who provide written consent again will be the target of a Phase II Second-Year Follow-up Study [4070-CL-0021] that is planned to be implemented.

3.3 Randomization

The treatment allocation manager will prepare the randomization list prior to the initial vaccination of the study drug to the first subject, which randomly allocates subjects to three groups in a ratio of 1:1:1 to ASP4070 4 mg group, ASP4070 1 mg group, or placebo group, with two factors as stratification factors, “Class of Japanese red cedar (JRC) pollen-specific IgE antibody test results at Screening visit 1” and “Change in the mean of total 3 nasal symptom (sneezing, nasal discharge, and nasal congestion) score during 120 to 180 minutes after start of cedar pollen exposure in the chamber as compared to the score before cedar pollen exposure at Screening visit 2.”

The person in charge of subject allocation at the study site will use the randomization list prepared by the treatment allocation manager to conduct allocation. Subject randomization will be conducted several times at the study site and completed before the initial study drug vaccination of subjects.

Specific procedures are provided in the “procedures for blinding compliance.”

4 SAMPLE SIZE

4.1 Primary Study Period

A total of 150 subjects, with 50 study drug vaccination subjects in each group

[Rationale]

The number of subjects required for 80% probability of detecting superiority of the ASP4070 group over the placebo group was calculated. Among the study results of other drugs targeting patients with cedar pollinosis using a chamber, studies with published data that allow the effect size to be calculated were confirmed and the effect size was 0.668 [a)], 0.903 [b)], and 0.962 [c)] for bilastine [Hashiguchi et al., 2017] , montelukast [Hashiguchi et al., 2012], and levocetirizine [Hashiguchi et al., 2013], respectively. Based on these effect sizes, with regard to the effect on the primary endpoint, the change in the mean of total 3 nasal symptom (sneezing, nasal discharge, and nasal congestion) score (3TNSS) during 120 to 180 minutes

after entering the chamber to the score before cedar pollen exposure, the effect size of ASP4070 was assumed to be 0.650. Using the significance level of the test to be 5% two-sided, when a hierarchical procedure is used for comparison with the placebo group in the order of high-dose group to low-dose group, if the effect size 0.650 is the same for the low-dose group as for the high-dose group, then the probability of detecting superiority of the low-dose group against the placebo group was calculated by simulation. Assuming the standard deviation to be 1, as a result of 10,000 simulations, the number of subjects required to ensure a detection power of over 80% was 48 subjects. Considering dropout cases, the number of subjects in each group was set to 50 subjects and 150 subjects in total.

a) With regard to the total 4 nasal symptom score (sneezing, nasal discharge, nasal congestion, and itchy nose) from 0 to 240 minutes after entering the environmental exposure chamber on Day 2 of administration, the mean for the bilastine 20 mg group and placebo group was 84.4 and 109.5, respectively, the standard deviation was 33.0 and 41.6, respectively, and the effect size was 0.668.
b) With regard to the mean of the total 3 nasal symptom score (sneezing, nasal discharge, and nasal congestion) from 120 to 180 minutes after entering the environmental exposure chamber, the mean of the montelukast 7 days group and placebo group was 2.31 and 3.17, respectively, the standard deviation was 0.17 and 0.20, respectively, and the effect size was 0.903.
c) With regard to the AUC of the total 4 nasal symptom score (sneezing, nasal discharge, nasal congestion, itchy nose) from 0 to 180 minutes after entering the environmental exposure chamber, the mean of the levocetirizine single agent group and placebo group was 16.78 and 31.35, respectively, the standard deviation was 14.01 and 16.21, respectively, and the effect size was 0.962.

4.2 Pollinosis Symptoms Survey Period and Additional Study Period

No statistical calculation has been made for the number of subjects in the pollinosis symptoms survey period and the additional study period. Subjects who have completed the primary study period will be included in the analysis for the pollinosis symptoms survey period, and subjects who are obtained informed consent for the additional study period will be included in the analysis for the additional study period.

5 ANALYSIS SETS

In accordance with International Conference on Harmonization (ICH) recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

The following policies apply to analysis sets in principle, but allocation of subjects to analysis sets is determined in the Classification Meeting by reference to opinions or advice of medical experts as needed.

5.1 Pollinosis Symptom Analysis Set (PSAS)

The Pollinosis Symptom Analysis Set will consist of all subjects who are randomized, are vaccinated with the study drug at least once, and have at least one measurement for an endpoint on pollinosis symptoms during the pollen dispersal season after vaccination of the study drug.

5.2 Immunological Analysis Set (IAS-2)

The immunological analysis set 2 (IAS-2) will consist of subjects who have provided additional consent for the additional test and have at least one measurement for immunological response endpoint during the additional study period.

6 ANALYSIS VARIABLES

6.1 Efficacy Endpoints

6.1.1 Pollinosis Symptoms Survey During 2018 JRC Pollen Dispersal Season

Endpoints during the pollinosis symptoms survey period (01 Feb 2018–31 Mar 2018):

6.1.1.1 Symptom Score

- Total nasal symptom medication score (TNSMS; range: 0-19)
- Total non-nasal symptom medication score (TNNSMS; range: 0-10)
- Total symptom medication score (TSMS; range: 0-29)
- Total nasal symptom score (TNSS; range: 0-12)
- Total non-nasal symptom score (TNNSS; range: 0-6)
- Total symptom score (TSS; range: 0-18)
- Individual nasal symptom (sneezing, nasal discharge, and nasal congestion) score
- Individual eye symptom (itchy eyes and watery eyes) score
- Individual medication (Tramazoline hydrochloride nasal drops, Ketotifen fumarate eye drops, and Fexofenadine hydrochloride) score
- Troubles with daily life
- Proportion of subjects who did not use rescue medications
- Proportion of subjects who used rescue medications within 7 days
- Proportion of days with each rescue medication
    - Tramazoline hydrochloride nasal drops
    - Ketotifen fumarate eye drops
    - Fexofenadine hydrochloride
- Proportion of days without Tramazoline hydrochloride nasal drops, Ketotifen fumarate eye drops, or Fexofenadine hydrochloride
- Proportion of days without Tramazoline hydrochloride nasal drops or Fexofenadine hydrochloride
- Proportion of days without nasal symptoms
- Proportion of well days
- Proportion of severe symptom days
- Proportion of subjects who achieved mean TNSMS in score <3, <4

For each symptom score, subjects will record the following items in their Pollinosis Symptoms Survey Diary every day. Section “12.3 Classification of Severity of Pollinosis Symptoms” in the protocol will be referred to with regard to nasal and eye symptoms and troubles with daily life.

- Nasal symptoms: Sneezing, nasal discharge, and nasal congestion, each graded with a 5-level score of 0, 1, 2, 3, and 4.
- Eye symptoms: Itchy eyes and watery eyes, each graded with a 4-level score of 0, 1, 2, and 3.
- Troubles with daily life: 5-level score of 0,1, 2, 3, and 4.

For total nasal symptom medication score, total eye symptom medication score, and total symptom medication score, usage of rescue medications will be scored and used for calculating the endpoints.

- Medication for nasal symptoms:
  - Usage of Tramazoline hydrochloride nasal drops, Fexofenadine hydrochloride tablets or other same kind of medication, each scored as 3. If either of Tramazoline hydrochloride nasal drops or Fexofenadine hydrochloride tablets is used, other same kind of medication will not be scored for the same day.
  - Usage of mask, scored as 1.
- Medication for eye symptoms:
  - Usage of Ketotifen fumarate eye drops or the same kind of medication, scored as 3. If Ketotifen fumarate eye drops is used, other same kind of medication will not be scored for the same day.
  - Usage of goggle, scored as 1.

Proportion of days with each rescue medication, proportion of days without rescue medications, proportion of days without nasal symptoms, proportion of well days and proportion of severe symptom days will be calculated for each subject. The denominator is the number of days the subject assessed. The day without nasal symptoms, the well day and the severe symptom day will be determined as below.

- Day without nasal symptoms: the day on which all of nasal symptom scores are 0
- Well day: the day on which all of nasal and eye symptom scores is 0 or 1 and no use of rescue medications
- Severe symptom day: the day on which either of nasal symptom scores is 4 or either of eye symptom scores is 3

6.1.1.2 Japanese Rhinoconjunctivitis Quality of Life Questionnaire: JRQLQ No 1

JRQLQ No 1 will be entered every 2 weeks during the pollinosis symptoms survey period for a total of 5 times. JRQLQ No 1 consists of the following three parts.

JRQLQ I (nasal and eye symptoms): each graded with 5-level score of 0 for NO SYMPTOMS, 1 for MILD, 2 for MODERATE, 3 for SEVERE and 4 for VERY SEVERE

- Runny nose
- Sneezing
- Blocked nose(nasal congestion)
- Itchy Nose
- Itchy Eyes

- Watery Eyes

JRQLQ II (QOL-related questionnaire): each graded with 5-level score of 0 for NO, 1 for SLIGHTLY, 2 for MODERATELY, 3 for GREATLY and 4 for VERY GREATLY

1. Reduced productivity at work/home
2. Poor mental concentration
3. Reduced thinking power
4. Impaired reading book/newspaper
5. Memory deterioration
6. Limitation of outdoor activity(e.g. sport, picnics)
7. Limitation of going out
8. Hesitation in communication
9. Difficulty in conversations and speaking over the phone.
10. Concerning people around
11. Impaired sleeping
12. Malaise
13. Fatigue
14. Low mood
15. Frustration
16. Depression
17. Dissatisfied with life

JRQLQ III (General State): graded with 5-level score from 0 for FINE to 4 for CRYING

The following variables will be calculated for analysis.

- Mean nasal and eye symptom score (Runny nose, Sneezing, Blocked nose(nasal congestion), Itchy Nose, Itchy Eyes and Watery Eyes)
- Mean nasal symptom score (Runny nose, Sneezing, Blocked nose(nasal congestion), and Itchy Nose)
- Mean eye symptom score (Itchy Eyes and Watery Eyes)
- Mean 3 nasal and 2 eye symptom score (Runny nose, Sneezing, Blocked nose(nasal congestion), Itchy Eyes and Watery Eyes)
- Mean score for each domain in the JRQLQ II
    - Usual Daily Activity: mean of items 1.-5.
    - Outdoor Activity: mean of items 6.-7.
    - Social Functioning: mean of items 8.-10.
    - Sleep Problem: item 11.
    - General Physical Problems: mean of items 12.-13.
    - Emotional Function: mean of items 14.-17.

6.1.1.3 Overall evaluation by the subject

Overall evaluation by the subject is conducted by subjects after the end of the pollinosis symptoms survey period.

- Overall evaluation by the subject: 5-level evaluation on overall evaluation of symptoms during the pollinosis symptoms survey period.

6.2 Immune Response Endpoints

Specific IgE antibody (anti JRC) and basophil activation test (BAT)

7 STATISTICAL METHODOLOGY

7.1 General Considerations

For continuous variables, descriptive statistics will include the number of subjects (n), mean, standard deviation, median, minimum, 25 percentile, 75 percentile and maximum. When needed, the use of other percentiles will be mentioned in the relevant section. Frequencies and percentages will be displayed for categorical data. Percentages by categories will be based on the number of subjects with no missing data, i.e. will add up to 100%.

All analyses will be presented by planned treatment group, unless specifically stated otherwise.

All statistical comparisons will be made using two sided tests at the α=0.05 significance level unless specifically stated otherwise. All null hypotheses will be of no treatment difference, all alternative hypotheses will be two-sided, unless specifically stated otherwise. No multiplicity adjustment will be done. All p-values will be presented as supportive information.

All data processing, summarization, and analyses will be performed using SAS Drug Development (ver. 4.5), and PC-SAS (ver. 9.4). Specifications for table, figures, and data listing formats can be found in the TLF specifications for this study.

For the definition of subgroups of interest please refer to section 7.8.

The coding dictionary for concomitant medication is WHODDE (B2) V2016MAR.

The values below the lower limit of quantitation (LLOQ) or above the upper limit of quantitation (ULOQ) will be treated as described in the table below when descriptive statistics are calculated.

Variable	Unit	LLOQ or ULOQ	Value used in analysis
Specific IgE Concentration (anti-JRC)	UA/ML	<0.10	0.10

7.2 Study Population

7.2.1 Disposition of Subjects

The following subject data will be presented:

- Number and percentage of subjects completed and discontinued in the pollinosis symptoms survey period, by primary reason for discontinuation for subjects who completed the primary study period, by treatment group and overall
- Number and percentage of subjects excluded from the PSAS by reason for exclusion, by treatment group and overall for subjects who completed the primary study period
- Number and percentage of subjects with sample taken for parameter for subjects with the informed consent for additional study period, by treatment group and overall
- Number and percentage of subjects excluded from the IAS-2 by reason for exclusion, by treatment group and overall for subjects with the informed consent for additional study period

7.2.2 Demographic and Other Baseline Characteristics

Demographic and other baseline characteristics will be summarized for the PSAS and IAS-2 by descriptive statistics.

The following variables will be summarized using descriptive statistics and frequency tabulations.

Variable	Statistics/Categories
Sex	Male, Female
Age at Informed Consent (years)	Descriptive Statistics
Age at Informed Consent (years) Group	>=20 to <30, >=30 to <40, >=40 to <50, and >=50
Height at Screening Visit 1 (cm)	Descriptive Statistics
Weight at Screening Visit 1 (kg)	Descriptive Statistics
Body Mass Index (BMI) at Screening Visit 1 (kg/m^2)	Descriptive Statistics
Duration of Disease (years)	<5, >=5 to <10, >=10 to <15, >=15 to <20, and >=20
Duration of Disease (years) Group	<10, >=10 to <20, and >=20
Severity of Pollinosis Symptoms in 2017	Good (Almost Asymptomatic), Slightly Good (Mild), Not Bad (Moderate), Slightly Bad (Severe), and Very Bad (Most Severe)
Severity of Pollinosis Symptoms in 2017	Good, Slightly Good or Not Bad,

Variable	Statistics/Categories
Group	Slightly Bad or Very Bad
JRC Specific IgE Antibody Test at Screening Visit 1 (UA/mL)	Descriptive Statistics
Class of JRC Specific IgE Antibody Test at Screening Visit 1	3, 4, 5 and 6
	3 and >=4[#1]
Change in the mean of 3TNSS during 120 to 180 minutes after start of cedar pollen exposure as compared to the score before cedar pollen exposure at Screening Visit 2	Descriptive Statistics
	<5 and >=5[#1]
Mean of TNSMS from 1 Feb 2018 to 7 Feb 2018 during the pollinosis symptoms survey period	Descriptive Statistics

#1: categories as stratification factors

7.3 Study Drugs

The following information on drug exposure will be presented by treatment group for the PSAS and IAS-2:

- Number and percentage of the number of vaccinations of study drug subject received
- Number and percentage of subjects receiving study drug at each visit

7.4 Analysis of Efficacy

7.4.1 Analysis of Pollinosis Symptoms Survey

All analysis of pollinosis symptoms survey will be presented by treatment group for the PSAS.

7.4.1.1 Symptom Score

Descriptive statistics will be presented for the following variables in 4 analysis periods A, B, C and D (refer to the section 7.11.4). TNSMS will also be summarized by the subgroup as described in the section 7.8 in detail.

Variable	Analysis Period
Mean TNSMS, TNNSMS, TSMS, TNSS, TNNSS and TSS	A, B, C and D
Mean nasal and eye symptom scores	A, B, C and D
Mean medication scores	A, B, C and D
Mean score for troubles with daily life	A, B, C and D
Proportion of days with each rescue medication	D
Proportion of days without Tramazoline hydrochloride nasal	D

drops, Ketotifen fumarate eye drops, or Fexofenadine hydrochloride	
Proportion of days without Tramazoline hydrochloride nasal drops or Fexofenadine hydrochloride	D
Proportion of days without nasal symptoms	D
Proportion of well days	A, B and C
Proportion of severe symptom days	A, B and C

Mean of TNSMS, TNNSMS, TSMS, TNSS, TNNSS, TSS, individual nasal and eye symptom scores, individual medication scores and score of troubles with daily life will be analyzed using an analysis of covariance (ANCOVA) model with treatment groups and the stratification factors as explanatory variables. The adjusted mean difference and its 95% confidence interval (CI) with p-values for ASP4070 group vs Placebo group will be presented. In addition, relative change will be calculated for reference.

Relative change (%) = (adjusted mean difference for ASP4070 group - Placebo group) / (adjusted mean for Placebo group calculated at mean values of covariates) x 100

<SAS code>

The following SAS code will be used for ANCOVA analysis:

```
PROC GLM;
  CLASS treatment strata;
  MODEL response=treatment strata;
  LSMEANS treatment / cl pdiff=control('placebo');
RUN;
```

where variables in the model are:

- treatment: Treatment group (ASP4070 4 mg, ASP4070 1 mg, and placebo)
- strata: Class of Japanese red cedar (JRC) pollen-specific IgE antibody test results at Screening visit 1 (3, >=4) and Change in the mean of 3TNSS during 120 to 180 minutes after start of cedar pollen exposure as compared to the score before cedar pollen exposure at Screening visit 2 (<5, >=5)
- response: Mean of TNSMS, TNNSMS, TSMS, 3TNSS, TNNSS, TSS, individual nasal and eye symptom scores, individual medication score and score of troubles with daily life

The day with each rescue medication, the day without rescue medications, the day without nasal symptoms, well day and severe symptom day will be defined for each day as a binary variable (Yes/No). Those binary variables will be analyzed using generalized linear mixed model with logit link function and factors for treatment groups and the stratification factors

as a fixed effect and subjects as a random effect. The adjusted odds ratio and its 95% CI with p-value for ASP4070 group vs Placebo group will be presented.

<SAS code>

The following SAS code will be used for analysis of generalized linear model with logit link:

```
PROC GLIMMIX method=RSPL;
  CLASS treatment strata;
  MODEL response=treatment strata / link=logit dist=bin ddfm=kr2 oddsratio;
  RANDOM int / subject=usubjid;
  LSMEANS treatment / cl pdiff oddsratio;
RUN;
```

where variables in the model are:

- treatment: Treatment group (ASP4070 4 mg, ASP4070 1 mg, and placebo)
- strata: Class of Japanese red cedar (JRC) pollen-specific IgE antibody test results at Screening visit 1 (3, >=4) and Change in the mean of 3TNSS during 120 to 180 minutes after start of cedar pollen exposure as compared to the score before cedar pollen exposure at Screening visit 2 (<5, >=5)
- usubjid: Subject Identifier
- response: Binary variable (Yes/No) for the day with rescue medication, the day without rescue medications, the day without nasal symptoms, well day and severe symptom day

Proportion of subjects who did not use rescue medications and proportion of subjects who used rescue medications within 7 days will be presented for the analysis period D by treatment group, along with its 95% confidence interval based on the Clopper-Pearson method. This endpoint will also be analyzed using a logistic regression model with factors for treatment groups and the stratification factors as explanatory variables. The adjusted odds ratio and its 95% CI with p-value for ASP4070 group vs Placebo group will be presented. In addition, adjusted difference in proportion between ASP4070 group and Placebo group and its 95% CI will be calculated using predicted values from the logistic regression model [Ge et al., 2011] (for SAS code, see also Appendix 1).

<SAS code>

The following SAS code will be used for logistic regression analysis:

```
PROC LOGISTIC;
  CLASS treatment strata;
  MODEL response=treatment strata;
```

RUN;

where variables in the model are:

- treatment: Treatment group (ASP4070 4 mg, ASP4070 1 mg, and placebo)
- strata: Class of Japanese red cedar (JRC) pollen-specific IgE antibody test results at Screening visit 1 (3, >=4) and Change in the mean of 3TNSS during 120 to 180 minutes after start of cedar pollen exposure as compared to the score before cedar pollen exposure at Screening visit 2 (<5, >=5)
- response: Binary variable (Yes/No) for subjects who did not use rescue medications and proportion of subjects who used rescue medications within 7 days

Proportion of subjects who achieved mean TNSMS for the period B in score <3 and <4 will be presented by treatment group, along with its 95% confidence interval based on the Clopper-Pearson method. This endpoint will also be analyzed using a logistic regression model with factors for treatment groups and the stratification factors as explanatory variables. The adjusted odds ratio and its 95% CI with p-value for ASP4070 group vs Placebo group will be presented. In addition, adjusted difference in proportion between ASP4070 group and Placebo group and its 95% CI will be calculated in the similar way as above.

As for TNSMS, TNNSMS, TSMS, TNSS, TNNSS, TSS, individual nasal and eye symptom scores, individual medication scores and score for troubles with daily life per day, the following data will be presented graphically by treatment group:

- Individual results using spaghetti plot
- Results using mean plot

In these plots JRC pollen counts will be overlaid on a right axis.

7.4.1.2 JRQLQ No 1

Results of JRQLQ No 1 will be summarized by treatment group and time point (1 Feb 2018, 17 Feb 2018, 3 Mar 2018, 17 Mar 2018 and 31 Mar 2018).

Mean nasal and eye symptom score, mean nasal symptom score, mean eye symptom score and mean 3 nasal and 2 eye symptom score in the JRQLQ I will be summarized using descriptive statistics.

Score for each questionnaire in the JRQLQ II will be summarized using descriptive statistics. Mean score for each domain will also be summarized.

Score for general state in the JRQLQ III will be summarized using descriptive statistics. Frequency tabulations for general state in the JRQLQ III will also be created.

The following data will be presented graphically by treatment group:

- Individual results using spaghetti plot
- Results using mean (+/- SD) plot

In these plots JRC pollen counts will be overlaid on a right axis.

7.4.1.3 Overall evaluation

Frequency tabulations for overall evaluation after the end of the pollinosis symptoms survey period will be presented. Shift table for overall evaluations from 2017 through 2018 will be presented by treatment group.

7.5 Analysis of Safety

Not applicable

7.6 Analysis of PK

Not applicable

7.7 Analysis of PD

Not applicable

7.8 Subgroups of Interest

In the analysis of symptom score, TNSMS will be summarized for the analysis period B using by treatment group for the subgroups defined on the categorized variables listed below:

Grouping Variable	Subgroups
Sex	Male Female
Age at Informed Consent (years)	>=20 to <30 >=30 to <40 >=40 to <50 >=50
Duration of Disease (years)	<10 >=10 to <20 >=20
Severity of Pollinosis Symptoms in 2017	Good, Slightly Good or Not Bad, Slightly Bad or Very Bad
Class of JRC Specific IgE Antibody Test at Screening Visit 1	3 >=4
Change in the mean of 3TNSS during 120 to 180 minutes after start of cedar pollen exposure as compared to the score before	<5 >=5

Grouping Variable	Subgroups
cedar pollen exposure at Screening Visit 2	

7.9 Other Analyses

7.9.1 Analyses of Immune Response

All analyses of immune response will be presented by treatment group for the IAS-2. The baseline visit is Day 1.

Descriptive statistics will be presented for quantitative variables (specific IgE antibody (anti-JRC) concentration and basophil activation test (BAT)) by treatment group and visit. Changes from baseline (Day 1) and percentage change from baseline will also be summarized. In addition, changes from Day 183 and percentage change from Day 183 will be summarized. As for specific IgE antibody (anti-JRC) concentration, percentage change from screening visit 1 to additional test will also be summarized.

Frequency tabulations will be presented for qualitative (specific IgE antibody (anti-JRC) class) variables by treatment group and visit.

The following data for quantitative variables will be presented graphically by treatment group:

- Mean (+/- SD) plot of actual values and change from baseline
- Individual results using spaghetti plot
- Individual changes from baseline using spaghetti plot
- Individual percentages change from baseline using spaghetti plot
- Individual changes from Day 183 to additional test in BAT using spaghetti plot
- Individual percentages change from Day 183 and screening visit 1 to additional test in specific IgE antibody (anti-JRC) concentration using spaghetti plot
- Box plot of percent change from Day 183 and screening visit 1 to additional test in specific IgE antibody (anti-JRC) concentration and change from Day 183 to additional test in BAT (activity 5ng/mL)

Scatter plot of the following variables on x-axis vs TNSMS for the analysis period B on y-axis will be presented:

x-axis (immune response endpoints)
Specific IgE antibody (anti-JRC) concentration at additional test
Percent change from baseline (Day 1), Day 183 and screening visit 1 to additional test in specific IgE antibody (anti-JRC) concentration
BAT (activity 5ng/mL) at additional test
Change from baseline (Day 1) and Day 183 to additional test in BAT (activity 5ng/mL)

7.10 Interim Analysis (and Early Discontinuation of the Clinical Study)

Not applicable

7.11 Handling of Missing Data, Outliers, Visit Windows, and Other Information

Whether to replace missing data and outliers and how to choose the analysis time points will eventually be decided by the database hard lock, taking the opinions and advice of medical experts into consideration as needed. Subjects or values excluded from analyses will be presented in listing of individual values but excluded in summarization such as summary statistics.

Refer to the data specification document in which more details are provided.

7.11.1 Missing Data

No imputation of missing data will be done.

7.11.2 Outliers

All values will be included in the analyses.

7.11.3 Visit Windows

The acceptable time ranges for the time points for analysis are as follows. If multiple data are obtained at the same time point, then data obtained on the closest day to the scheduled day will be used for analysis. If data are present before and after the scheduled day with the same day difference, then data obtained after the scheduled day will be used for analysis.
[Parameters]

Analysis Time Point	Scheduled day	Acceptable range
Screening visit 1	Date of informed consent	After date of informed consent until the day before Screening visit 2
Screening visit 3	28 days from Screening visit 2	28 days ±7 days from Screening visit 2 and also until Day 1 − 7 days
Day 1	Day 1	Until before study drug vaccination on Day 1
Day 57	Day 57	Within ±3 days of the scheduled day*
Day 127, Day 155, Day 183	Day 127, Day 155, Day 183	Within ±7 days of the scheduled day
Additional Test	16 May 2018	From 1 May 2018 to 31 May 2018

*: If the study drug is vaccinated, then data before study drug vaccination will be used.

7.11.4 Analysis Period for Pollinosis Symptoms

The analysis periods used for analysis of pollinosis symptoms survey are defined as follows:

Period	Description	Date (Duration)
A	Peak symptom period[*1] + 1 week pre- and post-peak symptom period	From 18 Mar. 2018 to 31 Mar. 2018 (14 days)
B[*2]	Peak symptom period for JRC pollen[*3] + 1 week pre- and post-peak symptom period	From 5 Mar. 2018 to 24 Mar. 2018 (20 days)
C[*4]	Peak JRC pollen dispersal period	From 24 Feb. 2018 to 31 Mar. 2018 (36 days)
D[*5]	Whole JRC pollen dispersal period	From 14 Feb. 2018 to 31 Mar. 2018 (46 days)

*1: Peak symptom period is defined as 7 consecutive days where the sum of daily average for TNSMS is the maximum during the pollinosis symptoms survey period (from 1 Feb. 2018 to 31 Mar. 2018). Sum of daily average is calculated per 1 day interval.

*2: To avoid results being confounded by Japanese cypress pollen, peak symptom period for JRC pollen + 1 week pre- and post-peak symptom period was completed before the first day (25 Mar. 2018) of the first two consecutive days which there was a cypress pollen count of >=30/cm^2/day.

*3: To avoid results being confounded by Japanese cypress pollen, peak symptom period for JRC pollen is defined as 7 consecutive days where the sum of daily average for TNSMS is the maximum from 1 Feb. 2018 until 24 Mar. 2018. Sum of daily average is calculated per 1 day interval.

*4: Peak JRC pollen dispersal period is defined as starting on the first day of the first two consecutive days during the pollinosis symptoms survey period (from 1 Feb. 2018 to 31 Mar. 2018) which there was a pollen count of >=30/cm^2/day, and ending on the last day on which there was a pollen count of >=30/cm^2/day.

*5: Whole JRC pollen dispersal period is defined as starting on the first day of the first two consecutive days during the pollinosis symptoms survey period (from 1 Feb. 2018 to 31 Mar. 2018) which there was a pollen count of >=1/cm^2/day, and ending on the day before the first of three consecutive days on which there was a pollen count of 0/cm^2/day.

8 DOCUMENT REVISION HISTORY

Version	Date	Changes	Comment/rationale for change
1.0	22-Jun-2018	NA	Document finalized

9 REFERENCES

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ICH Harmonized Tripartite Guideline E 3. Structure and Content of Clinical Study Reports, November 1995. (www.ich.org; Guidelines; "Efficacy" Topics)

ICH Harmonized Tripartite Guideline E 9. Statistical Principles for Clinical Trials, February 1998. (www.ich.org; Guidelines; "Efficacy" Topics)

Ge M, Durham LK, Meyer RD, et al. Covariate-adjusted difference in proportions from clinical trials using logistic regression and weighted risk differences. Drug Inf J 2011;45:481–93.

10 APPENDICES

10.1 Appendix 1: Sample SAS code for logistic regression

```
ods output ParameterEstimates=tmp2_2(keep=Estimate) CovB=tmp2_3;
proc logistic data=tmp1 outdesign=tmp2_1(drop=AVAL);
    class TRTPN(param=ref ref="3") STRATA1(param=ref ref="1") STRATA2(param=ref
ref="1");
    model AVAL(event="1")=TRTPN STRATA1 STRATA2/ covb;
run;

proc iml;
  use tmp2_1;
   read all into X;
  close tmp2_1;
  use tmp2_2;
   read all into Est;
  close tmp2_2;
  use tmp2_3;
   read all into CovB;
  close tmp2_3;

  n=nrow(X);

  X1=X[,1]||j(n,1,1)||j(n,1,0)||X[,4]||X[,5];
  X2=X[,1]||j(n,1,0)||j(n,1,1)||X[,4]||X[,5];
  X3=X[,1]||j(n,1,0)||j(n,1,0)||X[,4]||X[,5];

  XB1=X1*Est;
  XB2=X2*Est;
  XB3=X3*Est;

  P1=exp(XB1)/(1+exp(XB1));
```

```
P2=exp(XB2)/(1+exp(XB2));
P3=exp(XB3)/(1+exp(XB3));

A1=P1#(1-P1);
A2=P2#(1-P2);
A3=P3#(1-P3);

D1=t(A1)*X1/n;
D2=t(A2)*X2/n;
D3=t(A3)*X3/n;

dif1=sum(P1-P3)/n; * proportion difference(TRTPN=1 - TRTPN=3) ;
dif2=sum(P2-P3)/n; * proportion difference(TRTPN=2 - TRTPN=3) ;

se1=sqrt( D1*CovB*t(D1) + D3*CovB*t(D3) -2*D3*CovB*t(D1) ); * standard error of
proportion difference(TRTPN=1 - TRTPN=3) ;
se2=sqrt( D2*CovB*t(D2) + D3*CovB*t(D3) -2*D3*CovB*t(D2) ); * standard error of
proportion difference(TRTPN=2 - TRTPN=3) ;

upper1=dif1+se1*probit(1-0.025); * upper bound of 95% CI of proportion
difference(TRTPN=1 - TRTPN=3) ;
upper2=dif2+se2*probit(1-0.025); * upper bound of 95% CI of proportion
difference(TRTPN=2 - TRTPN=3) ;

lower1=dif1-se1*probit(1-0.025); * lower bound of 95% CI of proportion
difference(TRTPN=1 - TRTPN=3) ;
lower2=dif2-se2*probit(1-0.025); * lower bound of 95% CI of proportion
difference(TRTPN=2 - TRTPN=3) ;

create diff var {dif1 se1 upper1 lower1 dif2 se2 upper2 lower2};
  append;
close diff;
```

```
quit;
```

10.2 Appendix 2: Key Contributors and Approvers

List of Key Contributors and Approvers

Key Contributors

The following contributed to or reviewed this Statistical Analysis Plan as relevant to their indicated discipline or role.

Primary author (s)

[redacted]

Contributors and Reviewers

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