Statistical Analysis Plan

Prospective, randomized, double-blind, parallel, placebo-controlled clinical trial evaluating the safety, clinical efficacy, and immunomodulatory response of a polyvalent bacterial vaccine (Bactek®) administered via the sublingual mucosa in subjects with chronic obstructive pulmonary disease (COPD)

MV130-SLG-001

Statistical Analysis Plan

Version 1.0

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Abbreviations and Definitions

AE	Adverse Event
ARs	Adverse Reactions
COPD	Chronic Obstructive Pulmonary Disease
HGUGM	Hospital General Universitario Gregorio Marañón
HCSC	Hospital Clínico San Carlos
CRF	Case Report Form
CAT	COPD ASSESSMENT TEST
ELISA	Enzyme Linked ImmunoSorbent Assay
GCP	Good Clinical Practice
IgAs	Secretory Immunoglobulin A
IMP	Investigational Medical Product
ITT	Intention To Treat Set or Population
LNs	Local/regional mucosa-draining lymph Nodes
MALT	Mucosa-Associated Lymphoid Tissue
NKs	Natural Killer cells
PHA	Phytohemagglutinin
PIDs	Primary ImmunoDeficiencies
PP	Per Protocol Set or Population
RRTIs	Recurrent Respiratory Tract Infections
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SUSARs	Suspected Unexpected Serious Adverse Reactions

1. Revisions

[redacted]

2. Introduction

2.1. Preface

This statistical analysis plan (SAP) describes all planned analyses for the Clinical Study Report (CSR) of study MV130-SLG-001, a prospective, randomized, double-blind, parallel, placebo-controlled clinical trial evaluating the safety, clinical efficacy, and immunomodulatory response of a polyvalent bacterial vaccine (Bactek®) administered via the sublingual mucosa in subjects with chronic obstructive pulmonary disease (COPD).

The content of this SAP is based on protocol MV130-SLG-001 Amendment version 8. All decisions regarding final analysis, as defined in the SAP document, have been made prior to database lock of the study data.

Other endpoints, including immunological substudies, as well as other potential future analyses of data arising from this trial, will be the subject of future analysis plans.

2.2. Scope of the analyses

These analyses will assess the efficacy and safety of MV130 in comparison with placebo and will be included in the clinical study report. This statistical analysis plan (SAP) will give more detailed descriptions of the endpoints in the study and the corresponding analyses.

3. Trial Objectives

3.1. Primary Objective

The main objective of this trial is to evaluate the efficacy of a bacterial vaccine administered sublingually compared to placebo in subjects with moderate and severe COPD based on the number of COPD exacerbations.

3.2. Secondary Objectives

The secondary objective of this trial is to evaluate the impact of the investigational medicinal product on the following parameters:

- ★ Severity of the COPD exacerbations.
- ★ Time elapsed between the start of the treatment and the first COPD exacerbation.
- ★ Use of drugs (antibiotics, corticosteroids, etc).
- ★ Number of medical consultations due to COPD exacerbations.
- ★ Number of visits to the Emergency Department resulting or not in hospitalization.
- ★ Number of hospitalizations due to COPD exacerbations.
- ★ Days of hospitalization due to COPD exacerbations.
- ★ Health-related quality of life, as determined by an adapted version of a specific quality of life questionnaire: the COPD Assessment Test (CAT).
- ★ Healthcare expenditure resulting from resource consumption during episodes of COPD exacerbations occurring during the trial period.
- ★ Variations (change between the baseline level and months 0, 3, 6, and 12, as well as in comparison with the placebo) in the following immunological parameters in the patients participating in the substudy (N = 60) and/or those of the induced sputum study (N = 20) (between month 0 prior to the administration of the vaccine and month 12):
  - ➢ Specific humoral response [redacted]
  - ➢ Specific cellular response [redacted]

In a subgroup of patients (n = 20) selected from the group of patients participating in the immunological substudy (n = 60), a genetic study consisting in an evaluation performed at month (M) 0 (visit 1) and month 12 following the start of the vaccination will be performed on samples of induced sputum to study the following:

- ★ mRNA and miRNA expression profiles in PBMCs.
- ★ Proteomics using the iTRAQ technique.
- ★ Phenotypic markers of leukocyte populations.
- ★ Neutrophil function studies: phagocytosis (Phagotest) and bacteriophage capacity of the neutrophils (oxidative burst).
- ★ The first subgroup of subjects (N = 60) will be recruited from the [redacted] hospitals. The subgroup of subjects on which the induced sputum study will be performed (N = 20) (selected from the group of 60 subjects described above) will be recruited from the [redacted] hospital.
- ★ ▪ Safety outcomes
  - ➢ Overall rate, severity, and relationship of any adverse event (AE) per administration and patient.
  - ➢ Assessment of local tolerability (tissular reactions in the administration site).
  - ➢ Changes in standard laboratory parameters (serum chemistry and hematology).

4. Trial Design

This is a Phase II/III, prospective, multicenter, randomized, double-blind, parallel, placebo-controlled clinical trial where participants were administered sublingual bacterial vaccine or placebo during 12 months and were followed during an additional 6 months.

4.1. Study Assessments

Table 1 shows procedures to be followed during the study.

Table 1 Overview of the Trial Design, Procedures, and stages

VISIT No.	Baseline	1	2	3	4	5	6
Time (months since the start of the vaccination)		0	3	6	12	15	18
Period window			±10 days	±10 days	±10 days	±10 days	±10 days
Informed consent	✔						
Inclusion/exclusion criteria	✔						
Demographic data (including area of residence)	✔						
Vital signs (BP and HR)	✔						
Tobacco consumption (no. of cigarettes/day ✔ no. of years/20)	✔	✔	✔	✔	✔	✔	✔
Medical history/complete examination	✔	✔	✔	✔	✔	✔	✔
Exhaled CO	✔	✔	✔	✔	✔	✔	✔
Hematology	✔			✔	✔		✔
Serum chemistry	✔			✔	✔		✔
Viral serology testing (HBV, HCV, and HIV)	✔				✔		✔
Complete immunological evaluation	✔			✔	✔		✔
Immunological substudy (N = 60)		✔	✔	✔	✔		✔
Immunological substudy with induced sputum (N = 20)		✔			✔		
Spirometry	✔				✔	✔	✔
Recording of concomitant medication	✔	✔	✔	✔	✔	✔	✔
Pregnancy test	✔						
Recording of healthcare resource consumption	✔	✔	✔	✔	✔	✔	✔
Randomization no.		✔					
No. of days of antibiotic treatment required		✔	✔	✔	✔	✔	✔
Subject's visual analog scale score	✔	✔			✔	✔	✔
Investigator's visual analog scale score	✔	✔			✔	✔	✔

Quality of life questionnaire	✔	✔	✔	✔	✔	✔	✔
Provision of the patient diary		✔	✔	✔	✔		
Collection of the patient diary			✔	✔	✔		
Delivery of the sublingual vaccine		✔	✔	✔			
Collection of the sublingual vaccine			✔	✔	✔		
Recording of the no. of days of hospitalization		✔	✔	✔	✔	✔	✔
Recording of adverse events		✔	✔	✔	✔	✔	✔
Recording of unscheduled visits		✔	✔	✔	✔	✔	✔
Recording of trial dropout/termination cause		✔	✔	✔	✔		
Comments	✔	✔	✔	✔	✔	✔	✔
CRF review	✔	✔	✔	✔	✔	✔	✔

5. Trial Population

5.1. Population description

Three analysis sets are defined, the Full Analysis Set (FAS), the Per Protocol Analysis Set (PP) and the safety analysis set.

5.1.1. Safety Analysis Set

The Safety Analysis Set will consist of all subjects that are enrolled and receive at least 1 dose of MV-130. The safety analysis set comprises all randomized subjects.

5.1.2. Intention to Treat Set or Full Analysis Set (FAS)

All randomized subjects who have taken at least one dose. These subjects will be assigned to the corresponding treatment group.

5.1.3. Per Protocol Analysis Set (PP)

Randomized subjects completing the 50-week efficacy assessment period and adequately complying with the protocol. These subjects will be assigned to the actual treatment group. Any significant deviation from the treatment schedule will result in an exclusion from the "per-protocol" dataset.

5.2. Inclusion–Exclusion Criteria and General Study Population

A total of 198 subjects will be screened from the Respiratory Departments of participating centers.

These screened subjects will have been diagnosed with moderate or severe COPD.

5.2.1. Subject Inclusion Criteria

A COPD exacerbation will be considered to be recurrent when it occurs at a frequency of at least three episodes in one year.

In this study we will include subjects who meet the following criteria:

- ★ Subjects who have provided their written informed consent.
- ★ Subjects of both sexes.
- ★ Subjects aged between 35 and 85 years.
- ★ Subjects who are capable of complying with the dosing regimen.
- ★ Subjects with a diagnosis of moderate or severe COPD according to the GOLD criteria.
  - ➢ Subjects with a predicted post-bronchodilator force expiratory volume in the first second (FEV1) <50% (50% - 30%), with or without chronic symptoms (e.g., coughing or sputum production).
- ★ Subjects who have experienced at least three moderate exacerbations (i.e., those requiring treatment with antibiotics, systemic corticosteroids, or both, as prescribed by their general practitioner or pulmonologist in the standard consultation and/or the Emergency Department of their Clinic) or two exacerbations with at least one requiring hospitalization due to a COPD exacerbation and the other one a moderate exacerbation occurred within the last year.
- ★ Subjects who have not changed their medication for the maintenance treatment of COPD within the past 6 months.
- ★ Subjects with an accumulated consumption of ten or more pack-years. The subjects may or may not be active smokers.
- ★ Subjects included in the trial may have been vaccinated with pneumococcal polysaccharide vaccine at least 4 weeks before starting the administration of Bactek®.
- ★ Women of childbearing age must use an approved method of contraception (oral, vaginal, transdermal, intrauterine device [IUD], etc. or barrier methods) and obtain a negative result in the urine pregnancy test performed during the screening visit.

5.2.2. Subject Exclusion Criteria

- ★ Subjects outside the allowed age range.
- ★ Subjects who are unable to cooperate and/or have a severe psychiatric disorder.
- ★ Women who are pregnant, breastfeeding, expect to become pregnant during the study (including assisted reproduction), or who refuse to use contraceptives during the study (including barrier methods). Women who become pregnant during the

clinical trial will have to discontinue their participation in it.

★ Subjects who have participated in a study or clinical trial with an investigational product within the 3 months preceding their inclusion in this study.

★ Subjects diagnosed with asthma based on the guidelines of the American Thoracic Society and the European Respiratory Society. If the investigators are unable to differentiate between COPD and asthma after applying the criteria listed in the following table, a bronchodilator test with inhaled salbutamol must be performed, excluding those subjects with FEV1 changes >400 mL.

Medical History	COPD	ASTHMA
Smoker or ex-smoker	Almost all	Possible
Onset of symptoms <35 years	Rare	Common
Chronic productive cough	Common	Uncommon
Dyspnea	Persistent and progressive	Variable
Waking up in the middle of the night with dyspnea and wheezing noises	Uncommon	Common
Significant daytime or daily symptomatology changes	Uncommon	Common

★ Subjects with a diagnosis other than COPD that causes them to have an unstable condition or a life expectancy <3 years.

★ Subjects who experienced a COPD exacerbation within 4 weeks prior to the start of the trial.

★ Subjects with moderate COPD who required treatment with inhaled corticosteroids in the last 4 weeks.

★ Subjects with moderate COPD who received systemic corticosteroids (orally, intramuscularly, or intravenously) in the last 4 weeks.

★ Subjects diagnosed with a Primary (European Society for Immunodeficiencies [ESID] guidelines) or Secondary Immunodeficiency within the 12 months preceding their inclusion in the clinical trial or the trial's baseline visit.

★ Subjects diagnosed with a chronic lymphoproliferative disease.

★ Subjects diagnosed with a chronic infectious disease (tuberculosis [TB], HCV, HIV, or HBV).

★ Subjects with chronic heart disease, arrhythmias, or episodes of arrhythmia secondary to the use of bronchodilators.

★ Subjects diagnosed with COPD and chronic colonization by Pseudomonas aeruginosa.

★ Subjects with COPD and bronchiectasis diagnosed by CT imaging before the age of 40.

★ Subjects diagnosed with very severe COPD according to the GOLD classification.

★ Subjects requiring home oxygen therapy or non-invasive mechanical ventilation.

★ Subjects with a history of hypersensitivity to any of the vaccine's components.

★ Subjects receiving immunosuppressive treatment with: azathioprine, methotrexate, ciclosporin, cyclophosphamide, tacrolimus, antimalarial drugs, or gold salts.

★ Subjects who have been treated with monoclonal antibodies such as rituximab or TNF-alpha inhibitors in the last 6 months.

★ Subjects receiving chronic treatment with azithromycin or inhaled antibiotics (tobramycin or colistin).

6. Endpoints

6.1.1. Primary outcome

The primary efficacy outcome is a decrease in the mean number of COPD exacerbations. A clinical diagnosis of an exacerbation according to the GOLD definition will be considered; that is, an event occurring throughout the natural course of the disease, characterized by a change of acute onset, beyond the daily variability, in the patient's dyspnea, cough, and/or expectoration, which may require a change in the patient's usual medication.

6.1.2. Secondary outcomes

- ★ Decrease in the rate of COPD exacerbations per study group at 12 months (end of the trial treatment) and 6 months after the trial's termination (follow-up).
- ★ Decrease in the severity of the COPD exacerbations. The severity of the exacerbations will be measured based on the consumption of healthcare resources (i.e., visits to the Emergency Department, hospitalizations, or consultations), as follows:
  - ➢ ICU hospitalization: 4 points.
  - ➢ Standard hospitalization: 3 points.
  - ➢ Emergency Department visit: 2 points
  - ➢ Consultation involving a change in the patient's usual treatment: 1 point.
- ★ Time elapsed between the start of the treatment and the first COPD exacerbation
- ★ Use of drugs (antibiotics, corticosteroids, etc). Drug consumption will be scored as follows:
  - ➢ Use of antibiotics: 1 point.
  - ➢ Use of inhaled corticosteroids: 2 points.
  - ➢ Use of systemic corticosteroids: 3 points.
  - ➢ Use of oxygen therapy: 4 points.
  - ➢ Use of mechanical ventilation: 5 points.
- ★ Number of hospitalizations due to COPD exacerbations.
- ★ Days of hospitalization due to COPD exacerbations.
- ★ Number of visits to the Emergency Room.
- ★ Number of unscheduled medical consultations due to COPD exacerbations.
- ★ Health-related quality of life, as determined by an adapted version of the specific CAT test.
- ★ Healthcare expenditure resulting from resource consumption during episodes of COPD exacerbations occurring during the trial period.
- ★ Variations (change between the baseline level and months 0, 3, 6, and 12, as well as in comparison with the placebo) in the following immunological parameters in the patients participating in the immunological substudy (N = 60) and/or those of the subgroup of the induced sputum study (N = 20) (between month 0 prior to the administration of the vaccine and month 12).

In the case of patients participating in the IMMUNOLOGICAL SUBSTUDY (n = 60), changes in the below parameters will also be assessed during the randomization visit (visit 1) and the visits corresponding to months 3, 6, and 12:

- ➢ The specific humoral response [redacted]
- ➢ The in vitro proliferative response of specific T cells [redacted]

In a subgroup of patients (n = 20) selected from the group of patients participating in the immunological substudy (n = 60), the following parameters will also be evaluated in samples of induced sputum at month 0 (visit 1) and month 12:

- ★ IgA response to Bactek antigens assessed using ELISA technique.
- ★ Proteomic studies using the iTRAQ technique.
- ★ Genetic micro-RNA profile.
- ★ Phenotypic markers of leukocyte populations.
- ★ Neutrophil function studies: phagocytosis (Phagotest) and bacteriophage capacity of neutrophils (oxidative burst).

6.1.3. Safety outcomes

- ★ Overall rate, severity, and relationship of any adverse event per administration and patient.
- ★ Assessment of local tolerability (tissular reactions in the administration site).
- ★ Changes in standard laboratory parameters (serum chemistry and hematology).

7. Statistical Methods

All statistical analyses will be carried out by Inmunotek.

All computation will be performed according to European Guidelines

7.1. General Considerations

Descriptive statistics will be provided for selected demographic, safety, and exacerbations by dose, dose schedule, and time as appropriate. Descriptive statistics on continuous data will include means, medians, standard deviations (and standard errors for post-baseline data), quartiles, and ranges, while categorical data will be summarized using frequency counts and percentages. Graphical summaries of the data may also be presented.

When data are summarized by time, the values recorded against the scheduled time points listed in the protocol will be used. When assessing minimum/maximum increases or decreases over the study, all assessments, including unscheduled assessments will be used. Data listings will include all available data from all enrolled subjects unless specified otherwise.

This analysis does not use significance levels. Exact, two-sided 95% confidence intervals were used for the Histamine equivalent concentration.

7.1.1. Sample Size and Power

The calculation of the sample size is based on the efficacy measured based on the decrease in the number of episodes of COPD exacerbations during the 12-month treatment period of the trial per subject.

The ratio of subjects to be distributed among the active treatment and placebo groups will be 1:1.

We estimate that subjects from group I (active treatment with the bacterial vaccine) will achieve a 60% decrease in their number of COPD exacerbations compared with a 30% decrease among those of group II (placebo). With an alpha error of 0.05 and a beta error of 0.2, and assuming a dropout rate of 20-25%, the number of subjects to be recruited would be 90 per study group, with an overall total of 180 trial subjects.

7.1.2. Handling of Missing Data

No imputation of data will be carried out in case of missing data, but all available data will be used to its full extent.

7.1.3. Blinding

This is a prospective, multicenter, randomized, double-blind clinical trial comparing a treatment group with a placebo group. The ratio between the group receiving active treatment (group I) and the group receiving placebo (group II) is 1:1.

7.1.4. Randomization

Randomization will be done in blocks of 6, using a list of random numbers (randomization list) generated by the Head of the Clinical Epidemiology and Clinical Research Methodology Unit of Health Research Institute Hospital Universitario Clínico San Carlos, Madrid, Spain. A Stata user program was used to produce the randomization list.

The treatment assignment should always be carried out starting with the lowest number and progressing in a correlative way in increasing order.

7.1.5. Multiple Comparison

If multiple comparisons are needed, Holm adjustment for multiple comparisons will be used. However, the analysis will try to obviate multiple comparisons except as exploratory analysis.

7.1.6. Interim Analysis

There is no interim analysis planned.

7.1.7. Adjustments for Covariates

This study will use the draft guidance Adjusting for Covariates in Randomized Clinical Trials for Drugs and Biological Products - Guidance for Industry (CDER, May 2021, Revision 1). All baseline characteristics will be used to study exacerbations appearance in time using a Poisson regression or equivalent (see below).

7.1.8. Multicenter Trials
Statistical analysis will pool all available data from participating centers. There is no intention to compare centers in relation to the objectives of the study.

7.1.9. Model Assumptions
Primary endpoint analysis will use a median test or a Mann-Whitney test. Secondary endpoints will use relevant tests (Fisher's exact, T test, etc.) and a Poisson regression for exacerbations with baseline characteristics as covariates.

7.2. Subject Disposition
A table of subject disposition by treatment group displaying number and percent of subjects included in the FAS, in the PP analysis set and in the safety analysis set as well as number and percent of subjects enrolled and subjects withdrawn will be presented.

7.3. Demographic and Baseline Characteristics
Demographic and other baseline characteristics will be summarized displaying number of subjects, mean, standard deviation, median, 25 and 75-percentiles, minimum and maximum for continuous variables and frequency tables for categorical variables.

7.4. Efficacy Evaluation
The efficacy analyses will be conducted based on all the analysis sets.

7.4.1. Primary Efficacy Analysis
The rate of exacerbations per patient and the severity of these exacerbations will be compared using the chi-square test or Fisher's exact test. The relative risks will be estimated together with their 95% CI and median or Mann-Whitney tests. The number needed to treat (NNT) or number of events prevented per treatment will be estimated.

The rate of episodes occurred over time will be calculated, and the hazards ratio (HR) will be estimated together with its 95% CI following a Cox proportional-hazards model. The NNT (number of patients that need to be treated to avoid an event) will also be estimated.

Poisson model with robust estimation of incidence density will be used to include covariates in the model.

Poisson model assumes that the response variable is a count variable (number of exacerbations) and each subject has the same length of observation time. If the observation time for subjects varied, the Poisson model would need to be adjusted to account for the varying length of observation time per subject. In this case a Stata exposure option, exposure(*varname*), will be used where *varname* corresponds to the length of time an individual was followed to adjust the Poisson regression estimates. Also, Poisson model assumes that the dependent variable is not over-dispersed and does not have an excessive number of zeros (in whose case Negative Binomial and Zero-inflated models would be preferred).

7.4.2. Secondary Efficacy Analysis
The following variables will be analyzed to determine whether they follow a normal distribution: number of exacerbations, duration of the episodes, need for antibiotic therapy, number of hospital admissions, duration of the hospital admissions, immunological outcomes described in the trial design, quality of life, and direct costs.

These variables will be described together with their mean and standard deviation, or, in case of asymmetry, with their median and interquartile range. Differences between the means will be analyzed using Student's T test in the case of variables with a normal distribution and Mood's median test in the case of variables without a normal distribution. The absolute effect (difference in estimators) and its precision will be estimated with a 95% confidence interval (CI) in all cases.

The appearance of the first exacerbation will be studied at 3, 6, 12 months and whole study in the intention to treat (ITT) and per protocol (PP) sets and these same limits will be studied in the moderate and severe COPD populations. The appearance of any exacerbation at 3, 6, 12 months and whole study will be studied in the ITT and PP sets.

Number of exacerbations will be studied in the mild, moderate and severe COPD populations at at 3, 6, 12 months and whole study. Number of exacerbations in the ITT and PP sets will be studied at 3, 6, and 12 months.

To compare the change in the immunological variables described in the trial design at 0, 3, 6, 12, and 18 months, an analysis of covariance will be performed, estimating the mean difference over time in both study groups.

The cost-benefit ratios in the study groups will be estimated together with their 95% CI, and compared using non-parametric tests.

7.5. Safety Evaluation

All adverse events of the trial will be coded according to MedDra classification. MedDra version will be communicated in the statistical report. The safety variables will be compared using the chi-square test in the case of qualitative variables or Student's T test in the case of quantitative variables whenever they meet parametric assumptions, or using non-parametric test in the opposite case. AEs will be summarised by System Organ Class and Preferred Term displaying number of subjects, number and percentage of subjects having the event as well as number of events. Furthermore, the AEs will be summarised according to severity, relationship, outcome and seriousness.

7.6. Exploratory Analyses

This is the first trial with Bactek that will generate a lot of information that could be used in future research. For future research purposes only, exploratory analyses can be done outside of this SAP.

8. Deviations from the Trial Protocol

A secondary analysis will be conducted for the "Full Analysis Set" including all subject enrolled. Deviations will be listed and described.

Software to be used in the analysis

StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.

IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.

R Core Team (2022). R: A language and environment for statistical computing. R. Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

RStudio Team (2022). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.