CONFIDENTIAL

PROTOCOL No.: MV130-SLG-001

EUDRACT No.: 2012-003253-28

Version: 08.2 Date: 29/Dec/2017

INMUNOTEK, S.L.
Madrid, Spain
Medical Department

TITLE: 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)

Product:	BACTEK®
Indication:	Chronic Obstructive Pulmonary Disease
Country:	Spain
Route of administration:	Sublingual mucosa
Dosage:	See protocol
No. of subjects:	180
Trial duration:	7 years (estimated)

COORDINATING INVESTIGATOR

Dr. Eduardo Fernández-Cruz.

Clinical Immunology Service

Hospital General Universitario Gregorio Marañón

MEDICAL DIRECTOR

Dr. Miguel Casanovas

Inmunotek, S.L.

Version 01: 01/Jun/2012.
Version 02: this version incorporates clarifications from the Regional Clinical Research Ethics Committee (R-CREC) of the Autonomous Community of Madrid.
Version 03: this version incorporates modifications of relevant Amendment 01. Dated 21/Jun/2013.
Version 04: this version incorporates modifications of relevant Amendment 02. Dated 12/Feb/2014.
Version 05: this version incorporates modifications of relevant Amendment 03. Dated 10/Mar/2014.
Version 06: this version incorporates modifications of relevant Amendment 04. Dated 29/Oct/2014.
Version 07: this version incorporates modifications of relevant Amendment 05. Dated 26/Oct/2015.
Version 07.01: this version incorporates changes of the non-substantial modification. Dated 21/Jun/2016.
Version 08: this version incorporates changes of substantial modification 06. Dated 09/Jan/2017.
Version 08.1: this version incorporates changes of the non-substantial modification. Dated 21/Jun/2017.
Version 08.2: this version incorporates changes of the non-substantial modification. Dated 29/Dec/2017.

SIGNATURE SHEET

Protocol approval

Sponsor:

INMUNOTEK S.L

C/Punto Mobi, 5, 28805 Alcalá de Henares, Madrid, Spain

Telephone no.: [redacted] **Fax no.:** [redacted]

Sponsor's signature	**Signature**	**Date**
Dr. Miguel Casanovas	[redacted]	29/Dec/2017
Medical Director		

ANTONIO GALÁN SÁNCHEZ
Traductor-Intérprete Jurado de INGLÉS
Nº 9971

Dr. Eduardo Fernández-Cruz

Clinical Immunology Service

Hospital General Universitario Gregorio Marañón

Hereby declares:

That he has evaluated the protocol of clinical trial titled: "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)".

Sponsor's code: MV130-SLG-001 EudraCT No.: 2012-003253-28

Version: 08.2 Date: 29/Dec/2017

Sponsored by INMUNOTEK, S.L.

That the clinical trial respects the ethical standards applicable to this type of clinical trial.

That he agrees to participate in this clinical trial as the coordinating investigator.

That he has the necessary materials and human resources to carry out the clinical trial, without this interfering with the conduct of other types of trials or other tasks that are usually entrusted to him.

That he undertakes that each subject be treated and monitored in accordance with the provisions of the protocol, which was issued a favorable opinion by the Clinical Research Ethics Committee and authorized by the Spanish Agency of Medicines and Medical Devices (AEMPS, *Agencia Española de Medicamentos y Productos Sanitarios*).

That he will respect the ethical and legal standards applicable to this type of trial and will follow the Good Clinical Practice guidelines when conducting the trial.

That the collaborators that he needs to conduct the proposed clinical trial are suitable.

Signed in MADRID, Spain, on 29 December 2017

Signed: [Illegible signature]

Eduardo Fernández-Cruz: Coordinating Investigator

ANTONIO GALÁN SÁNCHEZ
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TABLE OF ABBREVIATIONS

AE	**A**dverse **E**vent
ARs	**A**dverse **R**eactions
COPD	**C**hronic **O**bstructive **P**ulmonary **D**isease
HGUGM	**H**ospital **G**eneral **U**niversitario **G**regorio **M**arañón
HCSC	**H**ospital **C**línico **S**an **C**arlos
CRF	**C**ase **R**eport **F**orm
CAT	**C**OPD **A**SSESMENT **T**EST
ELISA	**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay
GCP	**G**ood **C**linical **P**ractice
IgAs	**S**ecretory **I**mmuno**g**lobulin **A**
LNs	**L**ocal/regional mucosa-draining lymph **N**odes
MALT	**M**ucosa-**A**ssociated **L**ymphoid **T**issue
NKs	**N**atural **K**iller cells
PHA	**P**hyto**h**em**a**gglutinin
PIDs	**P**rimary **I**mmuno**D**eficiencies
RRTIs	**R**ecurrent **R**espiratory **T**ract **I**nfections
SAE	**S**erious **A**dverse **E**vent
SUSARs	**S**uspected **U**nexpected **S**erious **A**dverse **R**eactions

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1. TABLE OF CONTENTS

ANTONIO GALÁN SÁNCHEZ
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2. GENERAL INFORMATION

2.1. Protocol Title, Identification Number, and Date

- 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)
- Identification number: MV130-SLG-001
- Date: 29/Dec/2017
- Version: 08.2
- EudraCT No.: 2012-003253-28

2.2. Name and Address of the Sponsor and Monitor

- Name: Inmunotek, S.L.
- Address: Punto Mobi, 5, 28805 Alcalá de Henares, Madrid, Spain.

2.3. Name and Address of the Person Authorized by the Sponsor to Sign the Protocol and the Protocol Modifications

- Name: Dr. Miguel Casanovas.
- Position: Medical Director.
- Address: Punto Mobi, 5, 28805 Alcalá de Henares, Madrid, Spain.

2.4. Name, Title, Address, and Telephone Numbers of the Medical Experts of the Trial's Sponsor

- Name: Dr. José Luís Subiza. Immunologist.
  - Position: General Director.
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- Name: Dr. Miguel Casanovas. Allergologist.
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2.5. Name and Position of All the Investigators Responsible for Conducting the Trial, and Address and Telephone Numbers of the Trial Sites

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2.6. Name, Title, Address, and Telephone Number of the Person Responsible for Making the Medical Decisions Relating to the Trial Site

- Name: Dr. Javier de Miguel Díez.
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2.7. Name and Address of the Clinical Laboratory and Other Medical and/or Technical Departments and/or Institutions Involved in the Trial

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- Clinical Diagnosis Laboratory. Immunology Service. Hospital Universitario Clínico San Carlos of Madrid. Dr. [redacted]
  - Address: C/ Profesor Martín Lagos s/n 28040 Madrid, Spain.

The following tasks will be carried out:

Assessment of the following immunological parameters in patients participating in the study protocol, the "Immunological Substudy", and/or the induced sputum substudy (change between months 0, 1, 3, 6, and 12, as well as in comparison with the placebo) in the following immunological parameters:

General Immunological Studies:

- Specific humoral response (IgG serum concentration against Bactek® bacterial antigens; IgA concentration in patients' saliva and induced sputum against Bactek® antigens).
- Specific cellular response (specific CD4+ T-cell response in peripheral-blood mononuclear cells [PBMCs]) by means of a carboxyfluorescein diacetate succinimidyl ester (CFSE) assay after stimulation with the bacterial antigens that comprise Bactek®.

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- Genomics and Microarrays Unit. Hospital Clínico San Carlos of Madrid. Dr. Beatriz Pérez-Villamil and Laboratory of Clinical Molecular Immunogenetics. Clinical Immunology Service of HGUGM. Dr. [redacted]

  – Address: C/ Profesor Martín Lagos s/n 28040 Madrid, Spain.

  The following tasks will be carried out:

  – Assessment of messenger RNA (mRNA) and micro-RNA (miRNA) in PBMCs and induced sputum (change between the baseline level and months 1 and 12, as well as in comparison with the placebo).

  – Proteomics studies using the isobaric tags for relative and absolute quantification (iTRAQ) technique.

  – Phenotypic markers of leukocyte populations.

  – Neutrophil function studies: phagocytosis (Phagotest) and bacteriophage capacity of the neutrophils (oxidative burst).

- Statistics: [redacted]

  - Title: Head of the Clinical Epidemiology and Clinical Research Methodology Unit of Health Research Institute Hospital Universitario Clínico San Carlos.

  - Address: Calle del Profesor Martín Lagos 28040, Madrid, Spain.

  - Telephone no.: [redacted]

The following tasks will be performed:

– Epidemiological counseling.

– Preparation and custody of the randomization list.

- Entry of the data into the trial's database.
- Certification of the database lock.
- Preparation of the statistical plan.
- Statistical analysis.
- Preparation of the statistical report.

3. RATIONALE

3.1. Introduction

Healthcare focused on chronic infectious diseases involves a high consumption of the time of healthcare professionals and of the budget assigned by the Public Administration to the National Health System (NHS). Chronic obstructive pulmonary disease (COPD) is the most prevalent condition among such diseases, as recent epidemiological studies have estimated that over 10% of the adult population between the ages of 40 and 80 years is affected by this illness.[1, 2]

Approximately 85% of all cases of COPD are caused by smoking, and it is estimated that 15% of all smokers will develop COPD. Furthermore, because of the persistence of smoking among young people, the World Health Organization (WHO) considers that COPD will go from being the fourth cause of death worldwide to the third cause in 2020, following cerebrovascular disease and ischemic heart disease.[3]

Chronic obstructive pulmonary disease is a chronic and progressive disease characterized by frequent exacerbations during its clinical evolution. Flare-ups or exacerbations of COPD can be caused by a variety of risk factors, with recurrent bacterial and, predominantly, viral infections of the respiratory tract being most commonly associated with this condition and one of the most frequent causes of consultations in Primary Care centers and the hospital services of the NHS.[4]

This illness currently affects millions of people worldwide and is one of the leading causes of death with an increasing incidence.[5] In the USA, of the six most frequent causes of death, only the incidence of those due to COPD has risen constantly since the 1970s, which makes this condition the main cause of morbidity, mortality, and disability in this country[3] (Fig. 1). Although diagnosing this condition is not difficult, it is an underdiagnosed disease, particularly in the case of its milder and more treatable forms.[6] In 1993, the direct medical costs resulting from this disease in the USA summed up to 14.7 billion dollars, and the indirect costs related to its morbidity (e.g., loss of work days and productivity) were 9.2 billion dollars.[6]

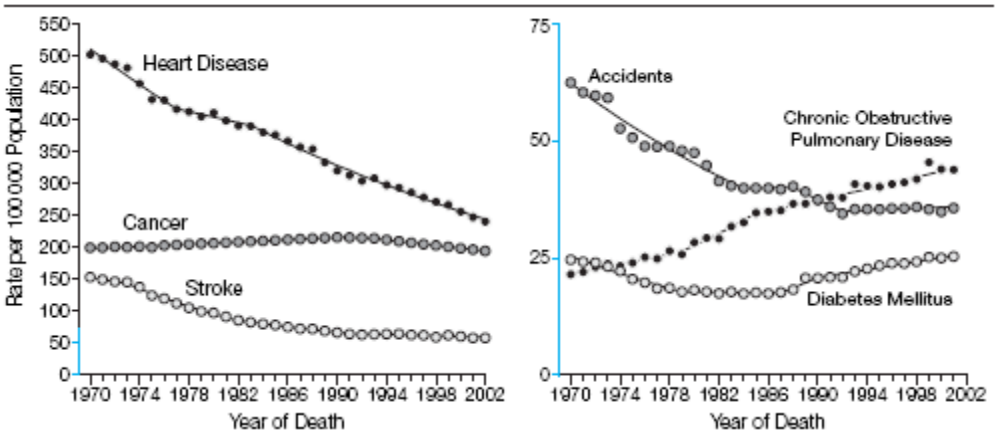

Figure 1. Deaths per 100,000 USA inhabitants resulting from the six most frequent causes of mortality throughout the 1970-2002 period.

In Spain, COPD affects 9% of people over the age of 40 and 20% of those over the age of 65.[7, 8] In a 2001 publication,[8] the authors estimated that, in Spain, COPD caused approximately 10-12% of consultations in primary care centers, 35-40% of pulmonology consultations, and 35% of permanent occupational disabilities. Moreover, the same authors indicated that COPD was responsible for 7% of hospital admissions and the fourth leading cause of death (33 deaths/100,000 inhabitants/year) in our country. The costs resulting from this disease were estimated to amount to 2% of the annual budget of the Spanish Ministry of Health and Consumer Affairs (*Ministerio de Sanidad y Consumo*) and to 0.25% of the gross domestic product. The annual social, occupational, and healthcare costs attributable to COPD were estimated at 2.4 billion euros, with an expenditure of 1,652-2,344 euros per patient and year. These costs have continued to rise as a result of the aging population, the increasing prevalence of this disease, and the price of new drugs and the latest therapeutic modalities.

3.2. Background of the Disease and Current Treatment Modalities

Albeit with significant seasonal variations, flare-ups or exacerbations of COPD represent 2% of all medical emergencies attended. Up to 40% of subjects presenting to a hospital Emergency Department due to an exacerbation of this illness have to be hospitalized. The consequences of severe COPD exacerbations are linked to high morbidity and mortality.[9] Frequent use of antibiotics for its treatment has been associated with increased antibiotic resistance.[10, 11] Exacerbations account for most of the healthcare costs associated with COPD.[12]

Current treatment modalities for COPD include a preventive approach in the early phase of the illness, including socio-environmental measures (primary prevention by promoting smoking cessation and physical exercise) and “opportunistic” screening interventions (secondary prevention by reducing underdiagnosis by means of the

early detection of the disease). In the phase in which the process has already begun, pharmacological treatment measures are implemented (antibiotic therapy, bronchodilators, and corticotherapy) and antigen-specific vaccines are administered (pneumococcal capsule polysaccharide vaccine and flu vaccine). The advanced or sequelae phase of COPD is characterized by a severe limitation of physical activity, generally accompanied by disability and depression, and response to treatment is only partial or incomplete.

Exacerbations are associated with a significant worsening in the patients' quality of life, and the degree of disability caused by this condition depends on the number of exacerbations experienced.[13] Albeit with seasonal variations, exacerbations of COPD are a frequent cause of consultations in both primary care and hospital services, and represent 2% of all medical emergencies attended. Exacerbations of infectious nature account for 1.5% of all emergencies attended in the hospital and for 13.7% of all infections. Up to 40% of subjects treated for this cause in the Emergency Department require hospitalization. Approximately 4% of the general European population consults their general practitioner at least once a year due to experiencing a respiratory exacerbation, and 20% of these consultations correspond to subjects with COPD. In Spain, empirical antibiotic treatment is prescribed in over 90% of cases of respiratory exacerbation of COPD, although sputum microbiology is only studied in 5% of subjects. Penicillins, cephalosporins, and macrolides are the most widely used antibiotics for the treatment of exacerbations of chronic bronchitis and COPD in Spain, followed by quinolones.[14]

Infection causes 75% of COPD exacerbations. Bacteria are responsible for half of these exacerbations of infectious etiology, which are mainly caused by *Haemophilus influenzae, Streptococcus pneumoniae*, *Moraxella catarrhalis,* and *Chlamydia pneumoniae*. However, in patients suffering from severe COPD presenting

with an exacerbation requiring mechanical ventilation, this infection might be caused by *Pseudomonas aeruginosa*. The rest of infectious exacerbations are caused by viruses, occasionally associated with bacteria, or exceptionally by other microorganisms.[14]

In addition to directly affecting social and health resources consumption, any intervention that can reduce the incidence of COPD exacerbations can have a great impact on the morbidity and quality of life of the affected subjects. Because bacterial infections of the respiratory tract are a well-known risk factor for the onset of COPD exacerbations, prevention and appropriate treatment of these infections may represent an attractive modality for avoiding the development of exacerbations.

The approach of identifying preventive therapeutic alternatives to avoid the development of COPD exacerbations is highly justified by the fact that controlling COPD exacerbations entails great costs for the subjects affected by this condition, society as a whole, and the NHS. However, there is currently little evidence of the existence of treatment modalities that can prevent the onset of COPD exacerbations associated with recurrent respiratory tract infections (RRTIs).

Immunotherapy treatment with new bacterial immunogens has emerged in the last decade as a therapeutic alternative capable of stimulating innate and specific adaptive immunity of subjects with several diseases. Sublingual immunization has recently been proposed as an alternative modality of mucosal vaccination that offers greater advantages for immunization against antigens and allergens in the treatment of allergies and respiratory infections.[15-22] However, this alternative has previously only been addressed in very few clinical trials (see below), which is why this trial is aimed at examining the effect of immunotherapy with bacterial immunomodulators on the prevention of the onset of COPD exacerbations in adults.

We will now proceed to describe the scientific rationale for this treatment modality, as well as the results of a limited number of open-label, uncontrolled and/or double-blind, randomized trials including a small number of subjects and whose findings suggest that this type of immunotherapy might reduce the risk of onset of RRTIs, and, in turn, lead to a decrease in the incidence of COPD exacerbations.

3.3. Name and Description of the Investigational Product

Bactek® is the generic name of this bacterial vaccine manufactured [redacted] by Inmunotek, S.L. (Spain). It contains whole, inactivated bacteria and is prepared individually for each patient. The latest generation of this product is fundamentally characterized by three new features: 1) its adjuvant-like glycerol solution, 2) its content featuring a high concentration of the bacteria that are most frequently present in the respiratory tract of subjects with recurrent respiratory tract infections, and 3) its administration in spray format via the sublingual mucosa.

According to this Clinical Trial protocol, each patient will be administered the **Bactek®** vaccine containing the following concentration of inactivated bacteria [redacted] *Staphylococcus aureus* (15%), *Staphylococcus epidermidis* (15%), *Streptococcus pneumoniae* (60%), *Klebsiella pneumoniae* (4%), *Branhamella catarrhalis* (3%), and *Haemophilus influenzae* (3%).The excipients that it contains are: glycerol (50%), pineapple essence q.s. (1 ml), sodium chloride (9 mg/ml), and water for injection q.s. (1 ml). This is the most frequently used bacterial composition in subjects with recurrent respiratory tract infections.

The **placebo** is an identical solution to the investigational medicinal product, but without the active ingredient, which are the bacteria in this case. The placebo only contains 50% glycerol, pineapple essence q.s. 1 ml, sodium chloride 9 mg/ml, and water for injection q.s. 1 ml.

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3.4. Summary of Findings of Potential Clinical Significance Obtained in Non-Clinical Trials and of Findings Relevant to the Current Trial Obtained in Clinical Trials

3.4.1. Non-Clinical Studies

Mucosa-associated lymphoid tissue (MALT) is a major contributor to the body's innate and adaptive immune system.[19, 23, 24] Recent studies on animal research models suggest that the immunotherapy modality that uses the mucosal route (i.e., oral, sublingual, rectal, vaginal, and intranasal mucosal vaccines) for the administration of the antigen is a potentially safe and efficient approach for the induction of immune responses with potential for preventing infectious diseases caused by bacterial and viral pathogens that enter the body through the mucosal surfaces.[4, 18, 19]

The immunological responses induced in the mucosa constitute an early and important defense line against several pathogens: bacteria, viruses, and parasites. Sublingual vaccination specifically induces antigen-specific immune responses of both types: mediated by activated B cells, involving the production of secretory IgA (surface IgA), IgM, and IgG antibodies; and CD4+ and CD8+ T-cell mediated responses, involving the production of Th_2 (IL-4 and IL-10) and Th_1 interleukins (TGF-β, IL-12, and IFN-γ), not only at the local mucosal level, but in the systemic compartments and the mucosa of distant locations from the administration site.[23, 25, 26] The existence of a high number of antigen-presenting cells (APCs) such as dendritic cells (DCs) in the sublingual mucosa facilitates the uptake, processing, and subsequent presentation of the antigen to the CD4+ and CD8+ T cells in the drainage lymph nodes located near the administration site. Antigen-presenting cells (DCs and macrophages) present in the lymph nodes, intestine, and lungs control the activation, expansion, and survival of cells regulating different phenotypes and functions such as CD4+ effector helper T cells (Th_1), CD4+ T cells (Th_2-like) that antagonize Th_1 cells and produce IL-4 and

IL-10, CD4+ or CD8+ TGF-β-producing T cells (Th_3-like), and CD4+CD25+ regulatory T cells (Treg) that inhibit CD4+ T-cell proliferation.[27-33]

Both in enteric and respiratory models, as well as in relation to mucosal immunization, in addition to the induction of one of the main humoral defense mechanisms present in the mucosal surface (i.e., IgAs), the generation of responses mediated by IFN-γ-producing CD4+ T cells (Th_1) and cytotoxic responses mediated by CD8+ T lymphocytes (i.e., cytotoxic lymphocytes [CTLs]) that are key for protective immunity and the clearance of bacterial and viral pathogens have also been described.[34-40]

The innate immune system is made up of several effectors, which primarily include macrophages, dendritic cells, and natural killer (NK) cells. Natural killer T (NKT) cells (CD3+CD16+/CD56+) are an important subset of lymphocytes featuring characteristics of both T and NK cells. These cells are part of both the innate and adaptative immune systems (antigen-specific B and T cells and their clonal memory responses) and act as a bridge between both types of immunity (innate and adaptative). These NKT cells form a subset of cells that has been assigned a protective role in infections, cancer, and sarcoidosis, although it has recently been attributed an important role in the immunopathogenesis of chronic pulmonary diseases, such as asthma, COPD, and hypersensitivity pneumonitis.[41-45]

NKT cells have the ability to both promote and suppress immune responses through the activation of different subtypes with various functions. Activated CD4+ NKT cells produce both Th_1 (IFN-γ and TNFα) and Th_2 (IL-4, IL-10, and IL-13) cytokines, whereas CD4-CD8- (double negative) and CD4-CD8+ NKT cells produce Th_1 cytokines and may exhibit antigen-specific cytotoxic and NK-like activity.[43, 46, 47]

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It has been postulated that the innate immune system mediates acute responses against infectious agents, whereas an atypical adaptive response can cause a chronic inflammatory response. Thus, it has been suggested that infections by bacteria and/or viruses, such as the respiratory syncytial virus (RSV), might trigger a persistent acute innate immune response and eventually result in an atypical adaptive immune response causing a chronic inflammatory disorder, such as in the case of asthma and COPD.[48-50]

Chronic obstructive pulmonary disease, which is characterized by respiratory bronchiolitis, chronic bronchitis, and emphysema, is largely mediated by a Th_1 immune response triggered primarily by CD4-CD8+ NKT cells (increased concentration of CD3+CD56+ CD1d-dependent NKT-like cells and decreased proportion of CD4+ NKT-like cells) selectively recruited in the lung and which, after interacting and activating macrophages, produce IL-13 and IL-17, consequently causing the chronic pro-inflammatory response observed in the COPD lungs.

In the local airway mucosa, the CD1d-expressing antigen-presenting dendritic cells can capture low amounts of the viral antigen and stimulate the activation of NKT cells. An experimental model of chronic pulmonary disease simulating COPD in humans showed that, after a viral respiratory infection, NKT cells (CD1d-dependent) migrate toward the lungs and activate chronically, resulting in an increased selective production of IL-13 mRNA that causes the recruitment and chronic activation of pulmonary macrophages (expressing CD1d), which, in turn, contribute to the predominant production of IL-13 mRNA in the lungs (in addition to the production of this cytokine by CD3+CD4+ T cells), which acts as the main mediator of the chronic inflammatory response. In the model, the presence of IL-13 is a requirement for the induction of the mucosa cell metaplasia and airway hyperreactivity that cause chronic inflammatory pulmonary disease after a viral infection. A similar phenomenon has

been observed in lung samples from COPD lung transplant recipients. Hence, this is the first description of an immune axis comprised by NKT cells, macrophages, and IL-13. Regulation of the NK cell function is a requirement for the optimal development of innate and adaptive immune antibacterial and antiviral responses.[41, 49, 51-53]

A crucial aim in vaccine development is the induction of an immunological memory originating a reservoir of effector cells with the ability to increase their number, as well as react quickly and effectively for prolonged periods of time, with specific immune responses generated when the presence of the specific pathogen is detected. The induction of this type of memory response is conditioned by the nature of the antigen, the adjuvant, and the route of administration. Thus, sublingual immunization would boast the advantage of modulating the generation of memory CD4+ helper T cells (Th_1) against specific inhaled pathogens by regulating the innate and adaptive antimicrobial defenses through the induction of humoral and cellular effector responses and maintaining a more effective, durable immune surveillance after the vaccination compared with other immunization routes.[15-22, 54-68]

3.4.2. Clinical Studies

The importance of considering the possibility of inducing memory immune responses at the mucosal level is explained by the fact that most human infections start or affect mucosal surfaces (e.g. gastrointestinal infections induced by *Escherichia coli*, *Clostridium difficile*, *Shigella*, rotavirus, etc.; respiratory infections induced by *Streptococcus pneumoniae*, *Haemophilus influenza*e, the influenza virus, the respiratory syncytial virus, etc.; or genital infections induced by the human immunodeficiency virus (HIV), the herpes simplex virus, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, etc.). In COPD, approximately half of exacerbations are associated with lower respiratory tract infections, mostly caused by common pathogenic bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, which accelerate the deterioration of pulmonary function in COPD. A functional defect in the innate immune response mediated by macrophages has been described in these subjects with COPD, who exhibit a decreased phagocytic ability to eliminate the cited pathogens and an altered cytokine production leading to bacterial colonization, chronic activation, and an increased frequency of exacerbations and deterioration of pulmonary function. As residents of the pulmonary mononuclear phagocytes, alveolar macrophages play a key role in the defense against inhaled pathogens.[69-73]

Immunomodulatory agents of diverse origin , including bacteria, can play an important role in the immunomodulation of the functional mechanisms of innate immunity mediated by NK cells, macrophages, and toll-like receptor (TLR2) molecules present on the surface of the alveolar macrophage whose expression and activation is involved in the recognition and phagocytosis of bacteria. Specific immunological functional defects against inhaled pathogenic bacteria are involved in the reduction

of bacterial clearance, thus leading to chronic infections that trigger exacerbations.[71, 74]

Bacterial immunomodulators can play a significant role in preventing infections that trigger such exacerbations.[75-78] Certain studies have demonstrated the efficacy of this type of product in the prevention of COPD exacerbations,[60, 79-81] some of them including a relatively significant sample size of subjects.[82]

A double-blind, placebo-controlled, randomized clinical trial (PARI-IS study) examined the effect of an immunomodulatory bacterial agent on the prevention of respiratory exacerbations in subjects with COPD. The results of this study showed that the immunomodulatory agent generated a clinical benefit in subjects with COPD by reducing the number of severe respiratory episodes requiring hospitalization.[60, 75, 76, 78, 82-87]

A recent systematic review analyzed the results obtained in 13 randomized, placebo-controlled clinical trials[83] including a total of 1971 subjects. The authors of the review acknowledged that two trials[82, 88] (including 731 subjects) followed the appropriate methodology and reported actions for the exacerbations, but that the rest of them were of low methodological quality and failed to conclusively demonstrate an effect on the prevention of exacerbations.

The immunomodulators used in all of these trials were bacterial lysates administered in capsules via oral swallowing. This oral route with distant mucosal stimulation, that is, at the level of the intestine, does not cause a systemic effect that is as effective as stimulation via the sublingual or nasal mucosal route.[15, 19]

A number of studies suggest that the clinical benefit may be greater when immunomodulation is achieved by direct stimulation of elements of the immune system present in the sublingual mucosa.[89, 90]

Overall, all studies[69, 72, 73, 91] suggest that immunomodulation via mucosal immunization with bacterial antigens can be considered a safe and effective immunotherapeutic delivery alternative for a robust and long-lasting stimulation of the innate immune system with simultaneous enhancement of local and systemic specific adaptative immunity. The enhancement of these immune responses has shown an association with the ability to induce resistance to infection and to physiologically modulate the chronic inflammatory response.

Therefore, these data strongly support the fact that immunization via the sublingual route can be an optimal option in terms of efficiency for the prevention of infections by inhaled respiratory bacteria. Moreover, it has not been demonstrated that the pharmacological agents currently available for the treatment of COPD (glucocorticoids, bronchodilators, azithromycin, and others) can exert a modulating effect on specific innate and adaptive responses of clinical relevance in the aberrant chronic inflammatory response associated with the progress of COPD.

Chronic obstructive pulmonary disease is characterized by the presence of acute exacerbations (COPD-AEs), which, along with its clinical symptoms and the subjects' spirometry results, define the risk of progression of the disease.[92] However, there are

no specific "biomarkers" that allow for characterizing the subgroup of patients suffering from more frequent exacerbations.

A number of studies have examined the inflammatory characteristics of sputum induced by hypertonic saline solution inhalation in patients with COPD, including its content in cells and inflammation-mediating molecules. However, there are currently very few biomarkers available for use in clinical settings for the assessment of COPD-AEs. In this study we are mainly interested in assessing the profile of the inflammatory cells present in the induced sputum of COPD patients. In this regard, there are a number of studies[93-95] that suggest that a target cell in COPD is the airway and lung neutrophil, which is functionally affected and one of the possible and important contributors to tissue inflammation, bacterial colonization, exacerbations, and progressive pulmonary function deterioration.[96-99]

In this disease, airway neutrophils are characterized by defective phagocytosis of pathogenic bacteria, a defective oxidative metabolism for the destruction of bacteria by an oxidative-dependent mechanism (oxidative burst), and an increased release of pro-inflammatory mediators such as cytokines and chemokines (IL-8, IL-6, and TNF-alpha) and proteolytic mediators (neutrophil elastase [NE], matrix metalloproteinase 9 [MMP-9], and myeloperoxidase [MPO]).[100, 101]

All of this leads to the patients' lungs being in a pro-inflammatory state (vicious cycle), associated with a dysfunction of the mucociliary system of the respiratory and cellular epithelium, a decrease in the alveolar phagocytic capacity, bacterial colonization, and damage to the lung tissue, which results in the development of an abnormal pro-inflammatory microenvironment in the lower respiratory tract that severely affects the clearance or elimination of bacterial pathogens.[69, 72, 73, 102, 103] Bacterial colonization of the lower airways induces chronic inflammation that contributes to their progressive obstruction by poorly-defined mechanisms.[104-106]

Our hypothesis is that BACTEK can immunomodulate phagocytic cell dysfunction and tissue inflammation in the lungs.

In this study, we aim to determine whether the neutrophil in the induced sputum can be used as a biomarker of altered innate immunity associated with the ability to eliminate the pathogenic bacteria that result in the pro-inflammatory state that causes the functional deterioration that characterizes patients with COPD-AEs.

3.4.2. Clinical Studies

A series of uncontrolled clinical studies on recurrent respiratory tract infections in adults and children have shown that the oral administration of polyvalent bacterial lysates improves clinical response by reducing the number, duration, and severity of infectious episodes.[78, 80, 82, 83, 107-110] A small number of uncontrolled clinical studies performed with COPD subjects who received polyvalent bacterial preparations (lysates) through the oral administration route suggest a beneficial effect in the prevention of disease exacerbations, with a decrease in the number, duration, and severity of infectious episodes and a reduction in antibiotic use and healthcare resource consumption reflected in lower rates of hospital admissions. In addition, these clinical trials have demonstrated a positive impact on impaired immune functions, such as alveolar macrophage activity and interferon gamma production. The usefulness of this therapeutic modality using bacterial lysates as immunomodulators in clinical practice has been a matter of debate due to the contradictory results observed in trials performed with the various types of bacterial preparations used in different clinical settings.

Several meta-analyses have been carried out on the use of various immunostimulants in respiratory infections:

- Bergemann *et al.* [85] carried out the first meta-analysis examining the efficacy of a bacterial lysate versus a placebo in reducing the number of acute exacerbations in subjects with chronic bronchitis, as well as a cost-effectiveness analysis.

- The meta-analysis performed by Del-Río-Navarro *et al.*[111] demonstrated that immunomodulators reduce the incidence of acute respiratory infections in children by an average of 40%. These investigators analyzed a total of 34 placebo-controlled trials with a total of 3877 participants.

- Schaad carried out a systematic review of the use of a bacterial lysate as an immunostimulant in pediatric subjects with recurrent respiratory tract infections.[112] Eight randomized, controlled studies were included in this meta-analysis, which showed that the population treated with this lysate developed significantly and consistently fewer cases of recurrent respiratory tract infections (26.2%) over a six-month period. The compiled data suggest that this effect is even greater in subjects at an increased risk of suffering from recurrent respiratory tract infections.

- Steurer-Stey *et al.*[83] carried out a systemic review analyzing the results of 13 randomized, placebo-controlled, clinical trials with a total of 1971 subjects. The authors of this review acknowledge that only two trials (including 731 subjects) followed the appropriate methodology and reported an effect on the exacerbations.

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~ Collet *et al.* performed a randomized, double-blind, placebo-controlled trial[113] to study the effect of an immunostimulant agent (consisting of a lyophilized lysate of eight of the most common pathogens isolated in respiratory tract infections and administered orally) to prevent acute airway exacerbations in subjects with COPD. In the same issue of the journal, Anthonisen published an editorial letter[76] praising the quality of the trial and pointing out that treatment with this bacterial lysate might be able to modify the course of COPD. This author also pointed out that, by halving the number of days of hospitalization, the costs resulting from this disease were very low, and made reference to the treatment's ease of administration.

~ Orcel *et al.*'s study[88] evaluated the preventive effects of oral vaccination with a bacterial lysate in elderly subjects with acute bronchitis. The results of this study suggested that the bacterial lysate exhibited a protective effect against acute bronchitis in elderly subjects.

– In a 2006 review of the Cochrane Collaboration,[114] six clinical trials comparing the effects of an *H. influenzae* vaccine in subjects with recurrent acute exacerbations of chronic bronchitis were analyzed. These six trials included a total of 440 subjects. The reviewers concluded that vaccinating subjects with recurrent acute exacerbations of chronic bronchitis in the fall season could reduce the number and severity of exacerbations during the following winter months.

– There are recent, very determining results about the effectiveness of immunological immunomodulators of bacterial origin administered via the sublingual mucosa in reducing respiratory infections in a population of susceptible subjects**.** A double-blind, placebo-controlled trial (PARI-IS study) evaluating the adjuvant effect of a bacterial immunogen in preventing

exacerbations in COPD subjects showed a reduction in the number of serious respiratory episodes requiring hospitalization.[82] Immunostimulation via the sublingual mucosa has shown to produce very intense innate and adaptive specific immune responses both locally and at a distant systemic level in the mucosa of the respiratory tract and lungs.[15, 19] There are also studies that show that whole inactivated bacteria result in a much more robust macrophage stimulation than that caused by bacterial lysates.[115, 116] However, there is still uncertainty about the type of innate and adaptive immune responses induced by these bacterial preparations that can effectively impact the clinical response of subjects with recurrent chronic respiratory tract infections.[77]

- Recent studies involving the use of bacterial vaccines have been conducted in subjects with recurrent respiratory infections [68, 78, 80, 108, 110, 117-123] and in subjects with COPD,[60, 75, 83, {Bourbeau, 2003 #14, 84, 87, 113, 124, 125] with their results suggesting their efficacy in reducing infectious episodes in these subjects.

- A pilot clinical trial with Bactek® has recently evaluated the effect of its daily administration via the sublingual route, for a total of six months, in the prevention of recurrent infections of the upper and lower respiratory tract (RRTIs) in a cohort of adult subjects.[126] This trial was carried out with a group of subjects with RRTIs (N = 17) to assess whether sublingual immunization with a polyvalent bacterial vaccine might cause an immunomodulatory effect on the antigen-specific immune response and has a clinical impact. An immunological assessment was performed both before and at the end of the immunization. These immunological determinations included: proliferation of antigen-specific CD3+CD4+ and CD3+CD8+ T cells against Bactek® antigens, serum immunoglobulin levels, specific antibodies against the pneumococcal

polysaccharide and the tetanus toxoid, and lymphocyte subpopulations of B, T, and NK cells. It has recently been published in the journal *Clinical Experimental Immunology*[126, 127] under the title: "Sublingual therapeutic immunization with a polyvalent bacterial preparation in patients with recurrent respiratory infections: immunomodulatory effect on antigen-specific CD4+ T cells and clinical impact".

During the twelve-month period elapsed since the start of the therapeutic immunization, an impact on clinical control was observed in the form of a significant decrease in the incidence of upper and lower respiratory tract infections compared with the number of RRTIs that the same subjects had experienced within the twelve months preceding the start of this treatment. This cohort of subjects exhibited a decrease in the frequency of episodes of RRTIs while receiving treatment with Bactek and during the follow-up period compared with the year preceding the start of the immunization. These clinical data are consistent with observations from another placebo-controlled study evaluating the bacterial vaccine in children, which showed that its clinical response is more effective in selected subjects who experienced a greater number of RRTIs in the year preceding the start of this therapy.[110]

The clinical trial with Bactek® demonstrated the existence of an association between a positive clinical response and an antigen-specific immune response mediated by CD4+ helper T cells.[127] A significant increase in the proliferative capacity of CD3+CD4+ T cells against Bactek® antigens was observed after 6 months of treatment with respect to the baseline (*P* <0.0001), and a significant increase in CD3+ T cells was also observed (*P* <0.05). No significant differences were observed in the serum levels of immunoglobulins, specific antibodies, or peripheral-blood lymphocyte subpopulations of B, T, and NK cells between the baseline and month 6. A significant reduction in the rate of respiratory infections was observed in the treated subjects compared with the rate of these infections during the year preceding the start of the immunization therapy (*P* <0.0001). The results of this study demonstrate that the long-term administration of a polyvalent bacterial preparation via the sublingual route in subjects with RRTIs exerts a stimulating effect on the immune response of CD4+ T cells against bacterial antigens, which could be associated with significant clinical benefits.

The significant increase in the proliferative capacity of CD3+CD4+ T cells could be one of the main immunomodulatory mechanisms at the systemic level induced by sublingual vaccination with the polyvalent bacterial preparation (Bactek®). However, the specific lymphoproliferative response is not the only immune mechanism that might have been modified in subjects by immunization with the bacterial antigens. It is possible that the innate immune response was stimulated locally, at the level of the different mucosal compartments of the upper and lower respiratory tracts, by immunomodulating the fundamental defect of the macrophage-mediated immune response against the respiratory bacterial pathogens, as well as that of other T, B, and NK-T effectors, thus normalizing the production of

pro-inflammatory cytokines in this microenvironment.[73] Stimulation of the innate response may have also contributed to the induction of the potent and sustained antigen-specific cellular immune response observed in immunized subjects.[48] However, the activation and interaction of the immunological effector mechanisms of the specific innate and adaptive systems need to be clarified, particularly in the context of their potentially important role in therapeutic immunomodulation and observed clinical improvement.[110]

An increase in the proliferative capacity of specific CD3+ CD4+ and CD3+CD8+ T cells against influenza virus antigens was also observed in this trial after 6 months of treatment with Bactek®.[12] The fact that the subjects had received the flu vaccine or had been infected by the influenza virus at least within the 2 years preceding the start of the treatment with Bactek® suggests that the bacterial immunization could have induced an immunostimulatory effect, which, in turn, resulted in the immunomodulation of the ongoing cellular immune response against the viral antigens.[128-132] This could be related to the existence of a dependence on the collaborative activity of the CD4+ T cells in increasing the number of antigen-specific CD4+ and CD8+ T cells[133] and to the observed generation and induction of specific CD4+ helper T-cell responses that have been linked to effective immune responses in long-term immunization therapies.[78, 107, 110]

The results of this pilot clinical study suggest a beneficial effect on the clinical response of RRTIs to a polyvalent bacterial vaccine administered sublingually and associated with an activation of the lymphoproliferative, antigen-specific CD4+ T-cell response against the bacterial antigens.[12] Overall these results are in line with those obtained in other studies that demonstrate an association

between immunization with polyvalent bacterial immunomodulators and the clinical improvement of respiratory diseases.[83, 108, 113, 124, 134, 135]

3.5. Summary of the Known Benefits for Human Subjects

This type of product has long been administered to humans as an individualized formula, with similar products also being administered to animals.

Several improvements derived from the knowledge obtained in studies on immunological mechanisms and leading to a greater clinical benefit have currently been introduced:

- This preparation is formulated with fully inactivated whole bacteria that induce a much more intense and robust macrophage stimulation response than bacterial lysates or receptor-specific ligands, owing to the fact that they produce a global stimulation of all receptors.[115, 116]

- This preparation is administered via direct deposition on the oral mucosa, mainly the sublingual one. This route of administration imitates the natural presentation of this type of antigen, as it involves the first mucosa that all antigens encounter when they enter the body through the respiratory and digestive systems. An extremely important component of the immune system, Waldeyer's tonsillar ring, is located in the orocervical region.[15, 19, 24]

- This preparation is administered as a spray in order to achieve a better dispersion over a large surface of the sublingual mucosa, thus allowing an improved uptake of the bacteria by the cells of the immune system.

- Given that it is administered as a spray, the patient has a lower sensation of deposited volume than if it were administered in the form of drops. In addition to increasing the area of the contact surface, this ensures that the subject will maintain contact between the preparation and the mucosa for longer.

- This preparation contains glycerol (50%), which provides greater stability, a bacteriostatic effect, and greater adhesion between the product and the mucosa.

- This preparation contains a pineapple flavoring agent that, together with the sweet taste of glycerol, helps to increase adherence and improved use of the preparation.

The main benefit offered by this therapeutic vaccination is the achievement of an adequate level of immunization against germs against which the subject's body is unable to defend itself effectively, which consequently causes recurrent infectious processes, usually focal or in areas limited to specific systems or organs, without achieving an adequate spontaneous immunization against them.

The fundamental advantage of the vaccine derives from its nature. It constitutes a preventive method that makes use of the body's natural and physiological mechanisms of anti-infectious defense, without introducing chemical elements that are foreign to the body, except for the immunogenic elements (bacterial antigens). It offers a second advantage over other immunization systems; that is, it precisely uses the germs responsible for the infections that are to be controlled. These germs are administered to the subject after their adequate processing and their inactivation.

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The main advantages of this preparation can be summarized in the following points:

- An immune response is obtained against the germs that are usually involved in the infectious processes that recurrently affect the subject.
- It reduces the overall use of antibiotics due to achieving an improved control of the infectious processes (number of cases and severity).
- It reduces the consumption of health resources, including medical actions (consultations, emergencies, hospitalizations, etc.), as well as ancillary examinations, which can sometimes be uncomfortable or risky, as well as repeated blood tests.
- It improves the patient's clinical condition.
- It confers a social and occupational advantage by reducing absenteeism.
- It is not associated with any added risks.
- It does not preclude the use of other treatments.
- Overall, it is a much cheaper therapy.

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3.6. Description and Rationale of the Route of Administration, Dosage, and Treatment Periods

3.6.1. Description and Rationale of the Route of Administration

This preparation (inactivated bacteria) is administered sublingually (oral mucosa) and initiates its effect on the immune system associated with said mucosa.

The mucosa that covers the respiratory, digestive, genitourinary, conjunctival, and auditory tracts, as well as the exocrine gland ducts, form a functional unit that, altogether, covers an area of approximately 300 m^2.

The mucosal immune system has the following basic functions[136]:

- To protect against pathogenic agents (anti-infectious effect).
- To act as a barrier against the penetration of infectious or immunogenic components present in the mucosa into the bloodstream and/or the body (barrier effect).
- To have a low reactivity against harmless antigens present on the mucosal surface (oral or mucosal tolerance).
- To maintain mucosal homeostasis (immunoregulatory function).

The basic characteristics of mucosal immunity that differentiate it from systemic immunity include:[136]

- A highly developed innate immunity.
-

- The existence of characteristic populations of lymphocytes and monocytes/macrophages in the mucosal compartments that differ from those of the peripheral blood in their phenotype and functional characteristics.

- The colonization of the mucosa and the exocrine glands by cells originating in lymphoid follicles (migration and settlement of mucosal lymphocytes, forming the so-called common mucosal immune system).

- Remote transport of polymeric immunoglobulins through the epithelium (secretory immunoglobulins).

Both the innate and acquired immune systems act in this common mucosal immune system,[19] where they feature inductive sites of innate immune response and effector sites of this response.[137] The inductive sites are formed by MALT and local/regional lymph nodes (LNs), whereas the effector sites are made up of different histological compartments, including the lamina propria of several types of mucosa, the stroma of exocrine glands, and the superficial epithelium[137] (Figure 2, from 137).

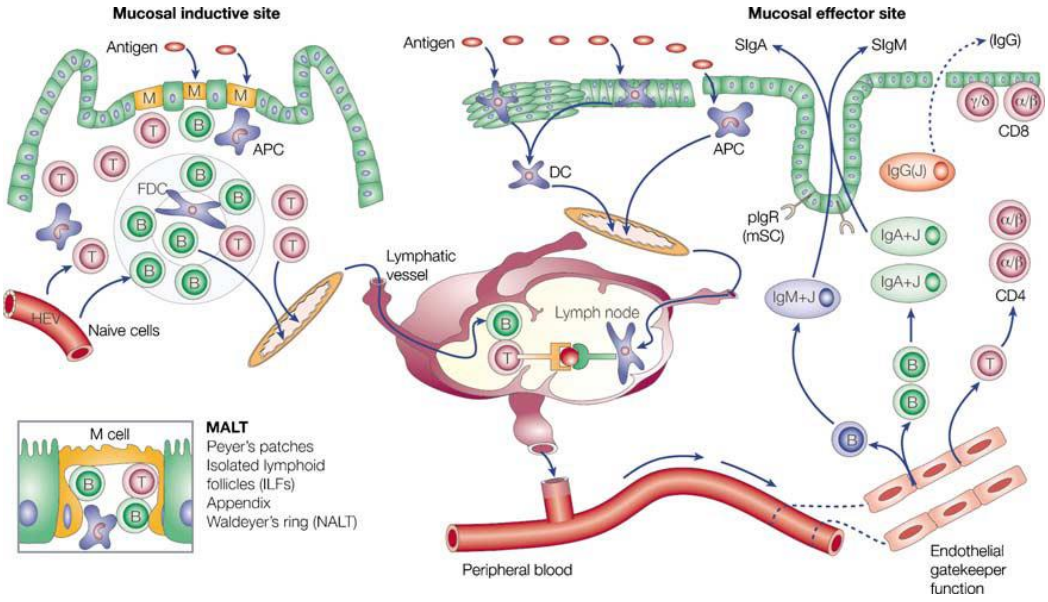

Figure 2. Representation of the mucosal immune system in humans

Stimulation of the oral mucosa can have an effect on distant mucosa[136] by activating the effector mechanisms of the innate and acquired immune systems. Figure 2 shows the expression of mucosal IgA in response to different routes of administration of the cholera toxin B subunit.[19] It should be noted that immunostimulation of the nasal mucosa is very effective in inducing the immune response in other types of mucosa. Oral/sublingual mucosal stimulation is as effective as nasal stimulation.[15] The efficacy and persistence of the immune response induced by sublingual immunization suggests that this route is a very promising alternative to immunization via other mucosal routes.[18]

The oral cavity harbours different types of mucosal tissue (masticatory, coating, and lingual).[138] It contains a high density of antigen-presenting cells, mainly Langerhans cells,[89] that boast a stimulating activity superior to that of the same cells present in the epidermis.[89] The presence of other antigen-presenting cells such as plasmacytoid dendritic cells is very low.[138]

Although the sublingual region exhibits the highest permeability,[139] other regions of the oral mucosa, such as the vestibular or cheek regions, also show an adequate degree of diffusion.[139, 140]

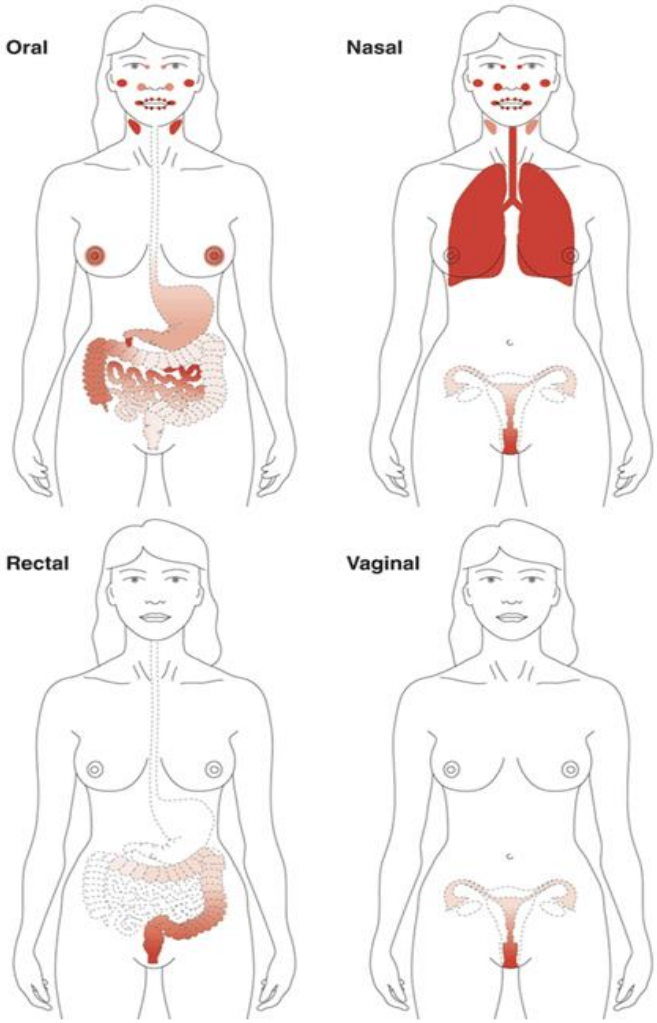

Figure 3. Production of IgA according to the type of mucosa stimulated (from 19)

There are several bacterial immunomodulators that contain inactivated bacteria, bacterial lysates, or cellular components of bacteria. Overall, they generate a non-specific stimulation of the innate and adaptive immune systems that affects both the cellular and humoral responses, thus offering a new avenue for establishing antimicrobial therapies, as they can stimulate the body's defense mechanisms and, as a result, prevent certain bacterial, viral, and fungal diseases.[107, 141-143]

3.6.2. Dosage

The treatment is presented in a bottle with an integrated spray dispenser that allows its contents to be distributed over a large surface. The standard dose is two puffs of the spray administered on the sublingual mucosa in the morning, on an empty stomach in order to avoid interferences with food. Each puff dispenses approximately 100 µl of liquid [redacted]

In patients diagnosed with severe COPD and who are receiving inhaled maintenance corticosteroid therapy (1-0-1), the investigational medicinal product must be administered in two sublingual puffs in the morning, on an empty stomach (before rinsing out the mouth with water) and two hours before the administration of the morning dose of the inhaled corticosteroids.

3.6.3. Treatment Period

The treatment period will last 12 months per subject. The treatment must be taken daily (2 puffs).

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3.7. Notification stating that this trial will be carried out in accordance with this protocol, the Good Clinical Practice Norms and the pertinent regulatory requirements.

This clinical trial will be carried out in accordance with the specifications set forth in this protocol and within the ethical and legal framework established by the Spanish Agency for Medicines and Medical Devices. Therefore, the following regulations will be applicable:

- Spanish Royal Decree 223/2004, of February 6th, regulating clinical trials with medicinal products and clarifications on the application of the clinical trial regulations as of 1st May 2004 (version no. 6, dated May 2008).
- Spanish Law 29/2006, of July 26th, on the guarantees and rational use of medicinal products and medical devices.
- Spanish Order SCO 256/2007, of February 5th, laying down the principles and detailed Good Clinical Practice guidelines and the requirements to authorize the manufacturing and importation of investigational drugs for human use (Official Spanish Gazzette [BOE, *Boletín Oficial del Estado*] no. 38, of February 13th) and its amendment through Order SCO/362/2008, of February 4th (BOE 41, February 16th).
- Good Clinical Practice Standards (International Council of Harmonization [ICH] E6: Good Clinical Practice: Consolidated Guideline, CPMP/ICH/135/95).
- Spanish Organic Law 15/1999 on the Protection of Personal Data.
- Basic Spanish Law 41/2002, of November 14th, regulating patient autonomy.
- The latest version of the Helsinki Declaration (59th WMA General Assembly, Seoul,

October 2008).

- The Oviedo Convention, of 4 April 1997, on human rights and biomedicine, ratified in the BOE of October 1999.

- The Eudralex European regulation on medicinal products, volume 10, on clinical trials:

- Directive 2001/20/EC of the European Parliament and the Council, of 4 April 2001, on the approximation of the laws, regulations, and administrative provisions of the Member States relating to the implementation of good clinical practices in the conduct of clinical trials with medicinal products for human use, aims to harmonize the laws of the Member States of the European Union in this respect.

- Directive 2001/83/EC, as modified by Directive 2003/63/EC.

- Directive 2005/28/EC lays down the principles and detailed GCP guidelines regarding investigational medicinal products for human use, as well as the requirements for authorizing the manufacture or importation of such medicinal products (transposed by ORDER SCO 256/2007, of February 5th).

- Spanish Royal Decree 1276/2011, of September 16th, governing the adaptation of regulations to the International Convention on the rights of people with disabilities that reforms Article 2.m of Spanish Royal Decree 223/2004 governing clinical trials, recognizes that people with disabilities who participate in clinical trials have the right to access information in appropriate formats, in accordance with the design criteria set out for all, and to have the necessary support in order for them to make their own decision, without prejudice to the provisions laid down for cases in which the person with a disability is legally incapacitated by a court ruling.

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By signing the protocol, the investigator commits to follow the instructions and procedures described in this protocol and the principles of Good Clinical Practice.

3.8. Description of the Study Population

Adult subjects (aged 35 to 85 years) with a clinical diagnosis of moderate or severe COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification.[92]

3.9. References to the Literature and Relevant Data for the Trial Providing Background Information About the Trial

It has been suggested that bacteria-derived products can act as non-specific immunomodulators by significantly increasing the innate immune response through toll-like receptors, the recognition of molecular patterns of bacterial nature, the activation of macrophages and the stimulation of their bacterial phagocytic and immunomodulatory activity by producing cytokines, and the activation of other effectors that might have a more specific reflection on the immune response.[107, 144, 145]

The bacterial vaccines that have been used thus far to treat recurrent respiratory tract infections contain various formulations of bacterial strains, including those that commonly infect the upper and lower respiratory tracts. These preparations can contain inactivated whole microorganisms, bacterial lysates, or several bacterial cell components.[107]

There are at least five medicinal products marketed in certain countries of the European Union that use bacteria, bacterial lysates, or bacterial fragments as immunomodulators in respiratory infections: Ismigen®, Respivax®, Broncho-Vaxom®, Ribomunyl®, and Luivac®.

The findings of the literature search relating to these products are detailed in the Investigator's Brochure.

Bactek® is a state-of-the-art bacterial vaccine that differs from other commercially available preparations in fundamental aspects that have been defined as crucial for the effectiveness of therapeutic immunization, such as its formulation (it uses whole inactivated bacteria instead of bacterial lysates and it contains glycerol as a excipient, which allows an optimal maintenance of its integrity) and route of administration (it is administered via the sublingual mucosa as opposed to the other routes, such as the oral, intranasal, and parenteral routes).

Several studies have recently been carried out on subjects with recurrent respiratory infections[68, 78, 80, 108, 110, 117-123] and COPD,[60, 75, 83, {Bourbeau,2003#14, 84, 87, 113, 124, 125] with their results demonstrating the efficacy of bacterial vaccines in reducing infectious episodes in these subjects.

A pilot clinical trial has recently evaluated the effect of the daily administration of Bactek®, via the sublingual route and for a total of six months, in the prevention of recurrent infections of the upper and lower respiratory tract (RRTIs) in a cohort of adult subjects.[126] This trial was carried out with a group of subjects with RRTIs to assess whether sublingual immunization with a polyvalent bacterial vaccine might cause an immunomodulatory effect on the antigen-specific immune response and its clinical impact. Seventeen subjects with RRTIs were recruited for this trial. They all received a daily administration of a polyvalent bacterial preparation (Bactek®) administered sublingually for a total of six months. An immunological assessment was performed both at the beginning and at the end of the immunization. These immunological determinations included: proliferation of antigen-specific CD3+CD4+ and CD3+CD8+ T cells against Bactek® antigens, serum immunoglobulin levels, specific antibodies against the pneumococcal polysaccharide and the tetanus toxoid, and

lymphocyte subpopulations of B, T, and NK cells. This study has been published in the journal *Clinical Experimental Immunology*[127] under the title "Sublingual therapeutic immunization with a polyvalent bacterial preparation in subjects with recurrent respiratory infections: immunomodulatory effect on antigen-specific CD4+ T cells and clinical impact".

During the twelve-month period of this study since the start of the therapeutic immunization, an impact on clinical control was observed in the form of a significant decrease in the incidence of upper and lower respiratory tract infections compared with the number of RRTIs that the same subjects had experienced within the twelve months preceding the start of this treatment. This cohort of subjects exhibited a decrease in the frequency of episodes of RRTIs while receiving treatment with Bactek and during the follow-up period compared with the year preceding the start of the immunization.

These clinical data are consistent with observations from another placebo-controlled study evaluating the bacterial vaccine in children, which showed that its clinical response was more effective in selected subjects who experienced a greater number of RRTIs in the year preceding the start of this therapy.[110]

The clinical trial with Bactek® demonstrated the existence of an association between clinical response and an antigen-specific immune response mediated by CD4+ helper T cells.[127] A significant increase in the proliferative capacity of CD3+CD4+ T cells against Bactek® antigens was observed at 6 months with respect to the baseline (P <0.0001), and a significant increase in CD3+ T cells was also observed (P <0.05). No significant differences were observed in the serum levels of immunoglobulins, specific antibodies, or lymphocyte subpopulations of B, T, and NK cells between the baseline and month 6. A significant reduction in the rate of respiratory infections was observed in the treated subjects compared with the rate of these infections during the year

preceding the start of the immunization therapy (*P* <0.0001). Hence, the results demonstrate that the long-term administration of a polyvalent bacterial preparation via the sublingual route in subjects with RRTIs exerts a stimulating effect on the immune response of CD4+ T cells against bacterial antigens, which could be associated with significant clinical benefits.

The significant increase in the proliferative capacity of CD3+CD4+ T cells could be one of the main immunomodulatory mechanisms at the systemic level induced by sublingual vaccination with the polyvalent bacterial preparation (Bactek®). However, the specific lymphoproliferative response is not the only immune mechanism that might have been modified in subjects by immunization with the bacterial antigens. It is possible that the innate immune response was stimulated locally, at the level of the different mucosal compartments of the upper and lower respiratory tracts, by immunomodulating the fundamental defect of the macrophage-mediated immune response against the respiratory bacterial pathogens, as well as that of other T, B, and NK-T effectors, thus normalizing the production of pro-inflammatory cytokines in this microenvironment.[48, 73] Stimulation of the innate response may have also contributed to the induction of the potent and sustained antigen-specific cellular immune response observed in immunized subjects.[48] However, the activation and interaction of the immunological effector mechanisms of the specific innate and adaptive systems need to be clarified, particularly in the context of their potentially important role in therapeutic immunomodulation and observed clinical improvement.[110]

An increase in the proliferative capacity of specific CD3+ CD4+ and CD3+CD8+ T cells against influenza virus antigens was also observed in this trial after 6 months of treatment with Bactek®.[12] The fact that the subjects had received the flu vaccine or had been infected by the influenza virus at least within the 2 years preceding the start of the treatment with Bactek® suggests that the bacterial immunization could have

induced an immunostimulatory effect, which, in turn, resulted in the immunomodulation of the ongoing cellular immune response against the viral antigens.[128-132] This could be related to the existence of a dependence on the collaborative activity of the CD4+ T cells in increasing the number of antigen-specific CD4+ and CD8+ T cells[133] and to the observed generation and induction of specific CD4+ helper T-cell responses that have been linked to effective immune responses in long-term immunization therapies.[77, 78, 107] The results of this pilot study suggest that the polyvalent bacterial preparation administered sublingually to subjects with RRTIs associated with an activation of the specific lymphoproliferative, antigen-specific CD4+ T-cell response has a beneficial effect on clinical response.

Overall, these results are in line with those obtained in other studies that demonstrate an association between immunization with polyvalent bacterial preparations and clinical improvement of respiratory diseases.[83, 108, 113, 124, 134, 135]

The **protocol** presented for this Clinical Trial is a prospective, randomized, double-blind, placebo-controlled study involving the use of a bacterial preparation (Bactek®) administered sublingually in a vulnerable population with a chronic respiratory tract disease (subjects with moderate and severe COPD). In this Clinical Trial we aim to prospectively determine whether daily sublingual immunization with Bactek® in subjects affected by this disease can generate a clinical benefit by preventing infections associated with COPD flare-ups or exacerbations by comparing them with a group of subjects receiving a placebo.

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We will perform a more thorough evaluation in a specific subgroup to determine whether the efficacy of this product in preventing infections is associated with the ability of the immunotherapy to induce a robust and sustained, local and systemic, innate and specific immune response at the level of the lungs by immunomodulating macrophages, specific CD4+ helper T cells, and the pro-inflammatory response.

Considering the high prevalence and cumulative high cost of the clinical management of chronic respiratory infections, as well as the frequent failure of conventional therapies, this new form of immunomodulation with bacteria could represent a new strategic design that is effective in reducing the cost, frequency, severity, and duration of COPD exacerbations.

4. OBJECTIVE AND PURPOSE OF THE TRIAL

4.1. Primary Objective of the Trial

- 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.

- 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 (concentration of serum IgG against Bactek® bacterial antigens and concentration of IgA in the patients' saliva and induced sputum) against Bactek® antigens.

  - Specific cellular response (specific CD4+ T-cell response in PBMCs) by means of a CFSE assay, after stimulation with the bacterial antigens that compose Bactek®.

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 HGUGM and HCSC 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 HGUGM 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).

5. TRIAL DESIGN

5.1. Description of the Primary and Secondary Outcome

- **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[92]; 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.[92, 146]

- **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.

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- 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 (concentration of serum IgG against Bactek® bacterial antigens and concentration of IgA against Bactek® antigens in the patients' saliva) assessed using the ELISA technique.

- The *in vitro* proliferative response of specific T cells (CD3+/CD4+ and CD3+/CD8+) after stimulation with the bacterial antigens that Bactek®comprises , assessed by means of CFSE staining and flow cytometry.

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).

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- 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).

5.2. Description of the Trial Type/Design

Prospective, multicenter, randomized, double-blind, parallel, placebo-controlled clinical trial.

Phase II/III.

Group I - The active group will receive the bacterial vaccine sublingually for 12 months.

Group II - The group that will receive placebo sublingually for 12 months.

5.2.1. Overview of the Trial Design, Procedures, and stages

Table 1

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	**X**						
Inclusion/exclusion criteria	**X**						
Demographic data (including area of residence)	**X**						
Vital signs (BP and HR)	**X**						
Tobacco consumption (no. of cigarettes/day x no. of years/20)	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Medical history/complete examination	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Exhaled CO	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Hematology	**X**			**X**	**X**		**X**
Serum chemistry	**X**			**X**	**X**		**X**
Viral serology testing (HBV, HCV, and HIV)	**X**				**X**		**X**
Complete immunological evaluation	**X**			**X**	**X**		**X**
Immunological substudy (N = 60)		**X**	**X**	**X**	**X**		**X**
Immunological substudy with induced sputum (N = 20)		**X**			**X**		
Spirometry	**X**				**X**	**X**	**X**
Recording of concomitant medication	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Pregnancy test	**X**						
Recording of healthcare resource consumption	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Randomization no.		**X**					
No. of days of antibiotic treatment required		**X**	**X**	**X**	**X**	**X**	**X**
Subject's visual analog scale score	**X**	**X**			**X**	**X**	**X**
Investigator's visual analog scale score	**X**	**X**			**X**	**X**	**X**
Quality of life questionnaire	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Provision of the patient diary		**X**	**X**	**X**	**X**		
Collection of the patient diary			**X**	**X**	**X**		
Delivery of the sublingual vaccine		**X**	**X**	**X**			
Collection of the sublingual vaccine			**X**	**X**	**X**		
Recording of the no. of days of hospitalization		**X**	**X**	**X**	**X**	**X**	**X**
Recording of adverse events		**X**	**X**	**X**	**X**	**X**	**X**
Recording of unscheduled visits		**X**	**X**	**X**	**X**	**X**	**X**
Recording of trial dropout/termination cause		**X**	**X**	**X**	**X**		
Comments	**X**	**X**	**X**	**X**	**X**	**X**	**X**
CRF review	**X**	**X**	**X**	**X**	**X**	**X**	**X**

Abbreviations: BP = blood pressure. HR = heart rate. CO = carbon monoxide. HBV = hepatitis B virus. HCV = hepatitis C virus. CRF = case report form.

Duration of the Trial

The trial is scheduled to comprise a 12-month treatment period and a 6-month post-immunization follow-up period (18 months total per subject). The trial shall end with the trial's database lock.

All subjects will start treatment with Bactek vs. placebo in a stable clinical condition.

- The subjects shall attend study visits every 3 months and whenever they develop symptoms of respiratory infection.
- They must strictly record the number of days of oral or systemic antibiotic treatment required until the remission of their ailment (COPD exacerbation).
- The number of days of hospitalization required due to the severity of the clinical condition (COPD exacerbation both during and after the immunization [6 months]) will be strictly recorded until the patient's clinical symptoms resolve and they are discharged home in a stable clinical condition.
- The pre- and post-immunization quality of life will be recorded using the specific CAT survey.

- Baseline Visit (prior to the start of the treatment)

  The baseline visit can take place 1-4 weeks before the start of the treatment (visit 1), and the following tests will be performed that day (Table 1):

  - Informed consent.

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- Inclusion and exclusion criteria.
- Demographic data (including residence data).
- Vital signs (body temperature [BT] and HR).
- Tobacco consumption (no. of cigarettes/day x no. of years/20).
- Medical history/complete examination (description of the abnormality).
- Measured exhaled CO.
- Collection of blood samples to perform the following tests:
  - ~ Hematology
  - ~ Serum chemistry
  - ~ Viral serology testing
  - ~ Immunological assessment

A blood sample of each patient will be collected during the baseline visit for the following:

- Determination of serum levels of IgG, IgA, and IgM. In patients with IgG levels <500 mg/dl or IgA levels <6 mg/dl, a determination of the IgG subclasses will be performed (nephelometry, Beckmann Coulter, USA) to rule out an immunodeficiency.

- Serum concentration of the anti-pneumococcal and anti-tetanus toxoid antibodies. An *in vivo* response study will also be performed in patients with low levels of antibodies in order to rule out an immunodeficiency in antibody

production.

- Spirometry.
- Recording of concomitant medication.
- Pregnancy test (for women of childbearing age).
- Recording of the consumption of healthcare resources.
- Subject's visual analog scale score.
- Investigator's visual analog scale score.
- Quality-of-life questionnaire.
- Comments.
- CRF review.
- **"IMMUNOLOGICAL SUBSTUDY":** variations in the below immunological parameters will be evaluated in a subgroup of patients (n = 60) during visit 1 (month 0, prior to the administration of the vaccine) and at months 3, 6, and 12:

    - The specific humoral response (concentration of serum IgG against Bactek® bacterial antigens and concentration of IgA against Bactek® antigens in the patients' saliva) assessed using the ELISA technique.
    - The *in vitro* proliferative response of specific T cells (CD3+/CD4+ and CD3+/CD8+) after stimulation with the bacterial antigens that compose Bactek®, assessed by means of CFSE staining and flow cytometry.

- Phenotypic flow cytometry study of the following PBMCs: CD19+ B, CD3+ T, CD4+ T, and CD8+ T lymphocytes; CD56+ NK cells; monocytes (CD11b/CD18); and NKT (CD1d and CD161) cells.

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

- IgA response to Bactek antigens assessed using ELISA technique.
- Capacity for the intracellular destruction of the previously opsonized viable bacteria contained in Bactek®, assessed based on the number of colony-forming units after incubation and lysis.
- Concentration of cytokines (TNFα, IFN- γ, IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, MCP-1, MIP-1α, IL-17, and IL-22) in the cell-free fluid, assessed using a multiplex system.
- Proteomics using the iTRAQ technique.
- Phenotypic markers of leukocyte populations.
- Neutrophil function studies: phagocytosis (Phagotest) and bacteriophage capacity of neutrophils (oxidative burst).

- Visits During the Trial

<u>First visit (month 0)</u>

- Randomization no. (only during visit 1).
- Tobacco consumption (no. of cigarettes/day x no. of years/20).
- Medical history/complete examination (description of the abnormality).
- Measured exhaled CO.

- Blood collection for the immunological substudy.
- Induced sputum collection in the case of patients included in the immunological substudy subgroup (N = 20).
- Registration of concomitant medication.
- Registration of health resource consumption.
- Recording of the number 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.
- Delivery of the sublingual vaccine (visit 1).
- Recording of the no. of days of hospitalization.
- Registration of adverse events.
- Registration of unscheduled visits.
- Recording of trial dropout/termination cause.
- Comments.
- CRF review.

The schedule of visits and evaluations to be performed during the treatment period are detailed in Table 1.

- Unscheduled visits:

  Trial subjects may attend an unscheduled visit whenever they experience a worsening of the disease under study.

  The tests/determinations performed during these visits will be recorded in the case report form.

- End of trial visit (see Table 1).

5.3. Description of the Measures Taken to Minimize/Avoid Bias

5.3.1. Randomization

Randomization will be done in blocks of 6, using a list of random numbers (randomization list) generated by the Head of the [redacted]

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

5.3.2. 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.

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5.4. Description of the Trial Treatment

5.4.1. Description of the Trial Treatment

The trial medication consists of an investigational medicinal product and a placebo. All patients included in the active group (Bactek®) of this trial will receive the same composition of Bactek® (described below).

- Investigational medicinal product:

  Polyvalent bacterial vaccine Bactek® contains different inactivated species (10^9 bacteria/ml) of bacteria that are frequently present in the respiratory tract:

  - *Staphylococcus aureus* 15%
  - *Staphylococcus epidermidis* 15%
  - *Streptococcus pneumoniae* 60% (containing a mixture of serotypes [redacted] in the same proportion)
  - *Klebsiella pneumoniae* 4%
  - *Branhamella catarrhalis* 3%
  - *Haemophilus influenza* 3%

It contains the following excipients: glycerol (50%), pineapple essence (q.s 1 ml), sodium chloride 9 mg/ml, and water for injection (q.s. 1 ml).

- Placebo

  Identical solution to that of the investigational medicinal product, but without the active ingredient (bacteria). It contains glycerol (50%), pineapple essence (q.s. 1 ml), sodium chloride (9 mg/ml), and water for injection (q.s. 1 ml).

5.4.2. Description of the Doses and Dosage Regimen of the Investigational Medicinal Product

The Investigational Medicinal Product must be administered exclusively via the sublingual route. The doses shall be administered below the tongue or on the cheeks (oral mucosa). The vaccine must be retained at this location, without swallowing it, for a total of 2 minutes, and then swallowed. The doses should preferably be administered on an empty stomach to avoid interferences with food.

The first dose shall be administered in the Hospital or Clinic in order to instruct the subject on the correct administration of the medication. The rest of the doses will be taken by the subject themself at home.

The established dosage regimen consists of the administration of two daily pulses in the sublingual region for a total of 12 months.

In patients diagnosed with severe COPD and who are receiving inhaled maintenance corticosteroid therapy (1-0-1), the investigational medicinal product must be administered in two sublingual puffs in the morning, on an empty stomach (before rinsing out the mouth with water) and two hours before the administration of the morning dose of the inhaled corticosteroids.

5.4.3. Description of the Pharmaceutical Form, Packaging, and Labeling of the Investigational Medicinal Product

The Technical Director of Inmunotek, S.L. guarantees the manufacture and adequate quality of the Investigational Medicinal Product in accordance with Annex 13 of the Good Manufacturing Practices guidelines.

The Investigational Medicinal Product will be supplied by Inmunotek, S.L. through the Hospital Pharmacy Department. The latter shall acknowledge receipt of the treatments in writing and be responsible for their correct storage and dispensing. Although the Investigational Medicinal Product remains stable at room temperature (25 °C), all supplies of this study medication should be stored in a refrigerated enclosure (between 2 °C and 8 °C). Access to the medication will be limited to the Principal Investigator, authorized members of their department, and the Pharmacy and/or nursing department.

Inmunotek S.L. will store the protocols concerning the manufacture and control of the batches of the products manufactured for the clinical trial in the master file, retaining samples of each batch up to twelve months after the expiry date.

The labeling of the samples for the clinical trial shall comply with the provisions of Annex 13 of the Good Manufacturing Practice guidelines for medicinal products in the European Union.

- The labels on the secondary packaging (box) will include the following information:

    a. Sponsor's name, address, and telephone number.

b. Pharmaceutical form, route of administration, and number of units.

c. Batch number or code identifying the contents and packaging operation.

d. Reference code allowing identification of the trial.

e. Trial subject identification number/treatment number.

f. Investigator's name (if not included in points a or d).

g. Instructions for use.

h. "Exclusively for use in clinical trials".

i. Storage conditions.

j. Expiry date in a month/year (MM/YYYY) format.

k. "Keep out of the reach of children".

- The labels on the primary packaging (bottles) will include the following information:

a. Sponsor's name: Inmunotek.

b. Pharmaceutical form, route of administration, and number of units.

c. Batch number or code identifying the contents and packaging operation.

d. Reference code allowing identification of the trial.

e. Trial subject identification number/treatment number.

The investigational medicinal product will be packaged in six boxes, each containing 2 or 3 bottles of the product, to be dispensed to each trial subject. All bottles contain the same concentration of the vaccine. During visit 1, the first box containing 3 bottles will be dispensed. During visit 2, boxes 2 and 3, both containing two bottles, will be dispensed and box 1 will be collected. During visit 3, boxes 4, 5, and 6, all containing 2 bottles, will be dispensed and boxes 2 and 3 will be collected. During visit 4, boxes 4, 5, and 6 will be collected.

The volume of leftover medicinal product contained within the returned vials will be measured to allow for assessing the degree of compliance with the prescribed dosing of the trial medication.

5.5. Expected Duration of the Trial. Description of the Sequence and Duration of the Trial Periods, Including Follow-up

Duration of the recruitment period: approximately 5 years. The recruitment period will end on 31/Mar/2018.

Total trial duration: 7 years (estimated duration).

The trial will last a total of 18 months per subject.

All subjects will be administered either the sublingual vaccine or a placebo for 12 months (52 weeks). The clinical response will be recorded every 3 months up to a maximum of 12 months (52 weeks), as well as 3 and 6 months following treatment completion (6-month follow-up period without vaccination).

5.6. Description of the Subject "Termination Criteria" and "Discontinuation Criteria" During the Entire Trial or Parts of It

The trial shall end with the trial's database lock.

Subjects may temporarily discontinue the treatment for 7 to 14 days in any of the following cases:

- Severe or very severe COPD exacerbation.

Acute exacerbations will be graded according to the following scale:

1. Mild (home care with or without contact with the subject's doctor).

2. Moderate (requiring a visit to the Emergency Department).

3. Severe (requiring hospitalization).

4. Very severe (requiring intubation and mechanical ventilation).

5.7. Investigational Medicinal Product Accounting Procedures

Both the investigational medicinal product and the placebo will be sent to the Hospital's Pharmacy Service following Inmunotek's receipt of the approval issued by both the CREC and the Spanish Agency of Medicines and Medical Devices.

The trial medication must be dispensed or administered in compliance with the procedures described in this document. Only subjects included in the trial may receive the trial medication in accordance with all applicable regulatory requirements.

The trial medication must be stored in a secure location until it is delivered to the trial subjects.

The principal investigator, or their designated person, will be responsible for the accounting of the trial medication, as well as for reconciling and storing the records

submitted by Inmunotek. This person will document the amount of medication received from Inmunotek, the amount dispensed to the trial subjects, and the amount returned by the trial subjects.

At the end of the trial, both the monitor and the Principal Investigator will create an inventory of both the unused medication and the medication returned by the trial subjects. This medication will be returned for its destruction to Inmunotek, S.L., as sponsor of the trial.

5.8. Maintenance of the Randomization Codes and Code Unlocking Procedures

In double-blind trials such as this one, the Investigator has a Code Unlocking mechanism available in case of emergency. This Code Unlocking should only be performed in case of emergency, whenever the Investigator needs to be aware of the identity of the trial drug in order to provide appropriate medical treatment or, when necessary, to ensure the safety of the trial participants. Inmunotek, S.L. must be informed immediately if a Code Unlock is executed for any subject. The reason for this Code Unlock must be documented on the cover or on the appropriate page of the CRF, along with the date and acronym of the person who unlocked the code.

5.9. Identification of any Datum to Be Recorded Directly in the CRFs and to Be Considered as an Original Datum

The visual quality-of-life scales will be completed directly in the CRF both by the subjects and the investigator and will be considered as source documents.

6. Screening and Withdrawal of Trial Subjects

A total of 180 subjects will be screened from the Pulmonology Services of Hospital General Universitario Gregorio Marañón, Hospital Clínico San Carlos of Madrid, Hospital Infanta Leonor, Hospital Universitario de Torrejón de Ardoz, Hospital

Universitario 12 de Octubre, Hospital Universitario La Paz, and Hospital Universitario de Vic.

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

6.1. Subject Inclusion Criteria

Recurrent respiratory infections are usually defined as those that occur at a frequency of at least three episodes in one year.[79, 147] The Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease[92] defines a COPD exacerbation as 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.[92, 146]

To unify the criteria, a COPD exacerbation will be considered to be recurrent when it occurs at a frequency of at least three episodes in one year.[79, 92, 146, 147]

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.[148]

  - ~ 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.

- The subjects included in the trial must live in the Autonomous Community of Madrid throughout the study period (September to May).

- 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.

6.2. 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.

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- 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).

6.3. Subject Withdrawal Criteria and Procedures

6.3.1. When and How to Withdraw Subjects from the Trial/Investigational Product

Any subject who has signed the informed consent and been assigned a randomization number will be considered a participant in the trial.

In the Informed Consent form, the subjects will be informed that they have the right to abandon the trial at any time without having to give any explanation and without any prejudice to them. This case will be considered as a voluntary dropout. In this event, the investigator must determine which of the following reasons is the main cause of such dropout:

- Any adverse event for which the investigator would not have deemed it necessary for the patient to abandon the trial.
- A concomitant disease.
- Withdrawal of the consent.
- Other causes.

Subjects who are withdrawn from the trial at the Investigator's discretion will be considered withdrawals. A withdrawal from the trial may be caused by any of the following circumstances:

- Treatment with a forbidden medication, as defined in this protocol.
- Deviation from the protocol, violation of the inclusion/exclusion criteria, or deviation from the treatment plan specified in this protocol (e.g., incorrect administration of the study medication or failure to attend the trial assessments).
- Pregnancy.
- If the Investigator deems that the subject's continued participation in the trial could be detrimental to their well-being.
- Intolerable adverse events at the investigator's and/or the subject's discretion.
- Poor compliance with the protocol or lack of treatment adherence.

The cause of the subject's dropout or withdrawal from the trial and the date on which it took place will be recorded in the CRF and the subject's medical record. In the event that the investigator recorded more than one cause for such dropout or withdrawal, they shall indicate which one was the main cause.

All cases of trial dropout caused by an adverse event shall be immediately notified to Inmunotek S.L, as the trial sponsor.

Any subject who drops out or is withdrawn from the trial before receiving the investigational medicinal product or the placebo will be considered a "screening failure".

6.3.2. Type of Data that will be Collected from the Withdrawn Subjects

All subjects who abandon the trial, regardless the cause, will be included in the statistical evaluation of the trial results, and the total number of withdrawals, the different causes of such withdrawals, and the moment they occurred in relation to the trial period must be recorded in detail.

No data will be collected for the statistical analysis from subjects considered to be screening failures.

6.3.3. Whether and How Subjects are to be Replaced

For the purposes of assessing the efficacy of the treatments under study, subjects whose cause of withdrawal is not related to any aspect of this trial (disease, medication, etc.) and whose withdrawal occurs before the expected six-month assessment, must be replaced.

6.3.4. Follow-up of Dropouts/Withdrawn Subjects

No follow-up will be carried out in the event of voluntary dropouts.

If the withdrawal is due to pregnancy, the subject will be followed to evaluate the outcome of the pregnancy (also including its early termination), and information about the mother's and child's condition will be sent to Inmunotek, S.L. The child will be followed for up to eight weeks after the delivery.

All cases of trial dropout caused by an adverse event shall be immediately notified to Inmunotek S.L, as the trial sponsor, who will follow the event according to the current legal regulations.

7. TREATMENT OF SUBJECTS

7.1. Treatment to be Administered, Including the Name of All Products and Doses

7.1.1. Treatments and Doses to be Administered

This trial will be performed with two parallel groups in a 1:1 ratio. Each group will receive one type of treatment.

Group I will receive an active treatment consisting of a bacterial vaccine administered sublingually.

Group II will receive a placebo as treatment.

7.1.2. Treatment Administration Schedule

The first dose of the treatment will be administered in the Hospital or the Clinic during visit one of the trial. The rest of the doses will be administered by the subject themself at home. The treatment will last 12 months per subject.

The treatment will consist in a total of 13 bottles that will be dispensed to each trial subject:

The investigational medicinal product will be provided in six boxes, each containing 2 or 3 bottles of the product, to be dispensed to each trial subject. All bottles contain the same concentration of the vaccine.

During visit 1, the first box containing 3 bottles will be dispensed.

During visit 2, boxes 2 and 3, both containing two bottles, will be dispensed and box 1 will be collected.

During visit 3, boxes 4, 5, and 6, all containing 2 bottles, will be dispensed and boxes 2 and 3 will be collected.

During visit 4, boxes 4, 5, and 6 will be collected.

7.1.3. Route/Mode of Administration of the Treatments to be Administered

The trial medication must be administered exclusively via the oral route (sublingually). The doses shall be administered below the tongue or on the cheeks. The vaccine must be held at this location, without swallowing it, for a total of 2 minutes, and then swallowed. The doses should preferably be administered on an empty stomach.

The first dose shall be administered in the Hospital or the Clinic in order to instruct the subject on the correct administration of the medication. The rest of the doses will be administered by the subject themself at home.

7.1.4. **Treatment Periods, Including the Follow-up Period**

The treatment and follow-up periods will jointly last 18 months (12 months of treatment and 6 months of post-vaccination follow-up without Bactek®).

7.2. Medication/Treatments Permitted (Including Rescue Medication) and Not Permitted Before and/or During the Trial

Permitted medication:

- All investigators agree to follow the COPD treatment guidelines as recommended by the GOLD[143] and that all subjects will be treated in the same way.
- Annual flu vaccine.

- Pneumococcal polysaccharide vaccine (>4 weeks before starting treatment with Bactek®).

7.3. Procedures for Monitoring Subject Compliance

The first sublingual dose will be administered in the Hospital, whereas the rest of the doses will be administered in the subjects' home.

In order to monitor treatment compliance, the subjects must:

- Complete and submit the medication log sheets provided to them.
- Attend the visits scheduled both for assessments and to dispense and collect the corresponding medication.
- Return the medication that they received during the previous visit.

To evaluate the degree of compliance with the prescribed dosing of the trial medication, the sponsor (Inmunotek, S.L.) will measure the volume of medication leftover in the vials that it will receive during each scheduled visit.

8. EFFICACY ASSESSMENT

8.1. Specification of Efficacy Parameters

- **Primary outcome:** the number of COPD exacerbations due to infectious episodes diagnosed by the clinician in charge.

- **Secondary outcomes**:

  - Rate of COPD exacerbations per patient included in the study.
  - Decrease in the 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 hospitalizations due to COPD exacerbations.
  - Days of hospitalization due to COPD exacerbations.
  - Number of visits to the Emergency Department resulting or not in hospitalization.
  - Health-related quality of life, as determined by an adapted version of the specific CAT test.
  - Ratio between the number of COPD exacerbations and the 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 immunological parameters described in page 63.
- 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).

8.2. Methods Used to Assess, Record, and Analyze the Efficacy Parameters

From the beginning of the trial, the subjects shall attend five scheduled visits (baseline visit, three follow-up visits, and the end of trial visit) with the study physician and unscheduled visits whenever their clinical condition warrants it.

During the baseline visit, the study doctor will conduct a detailed clinical interview during which the following will be recorded and performed:

- Screening of patients meeting the inclusion criteria.

- Signing of the informed consent form by the patient and the investigator.

- Collection of demographic data and ensuring that the patient will reside in the Autonomous Community of Madrid throughout the study period (September to May).

- Measurement and recording of vital signs (BP and HR).

- Collection of tobacco consumption data using the following formula: no. of cigarettes/day x no. of years/20 (baseline visit and visits 1, 2, 3, and 4).

- Testing for exhaled CO measure (all visits).

- Detailed medical history and general examination of the patient will be conducted, recording the data in the CRF (baseline visit and visits 1, 2, 3, and 4).

- Female subjects included in the study must provide a urine sample (first morning void) on which a pregnancy test will be performed based on a qualitative one-step chromatographic immunoassay (Acon) determining the presence of beta-human chorionic gonadotropin (beta-HCG). Subjects with a positive pregnancy test will not be included in the trial.

- Collection of blood from each subject (baseline visit, month 6, month 12, and end of trial visit at month 18) and analysis of the following parameters:

  - Complete blood count (flow cytometry).
  - Blood biochemistry, including basal blood glucose levels and liver function tests (enzymatic methods).
  - Viral serology testing: HCV, HBV, and HIV (ELISA serology).
  - General immunological parameters.

- All patients included in this trial will undergo a spirometry test during the baseline and end of trial visits (visits 4, at 12, 15, and 18 months from the start of the vaccination). Spirometry testing will be performed using the spirometer according to the protocol of the Respiratory Function Tests Unit of all hospitals participating in the trial.

- Any concomitant medication will be recorded in the CRF during all visits.

- The healthcare resources consumption will be recorded during all visits using the CRF.

- The number of days of antibiotic treatment required will be recorded during visits 1-4 using the CRF.

- A visual analog scale will be scored by the subjects during all visits and the result will be recorded in the CRF.

- A visual analog scale will be scored by the investigator during all visits and the result will be recorded in the CRF.

- The CAT Quality-of-Life Questionnaire will be completed during all visits.

- The Patient Diary will be provided during visits 1-4.

- The Patient Diary will be collected during visits 2-4.

- The sublingual vaccine will be dispensed during visits 1-3.

- The sublingual vaccine will be collected during visits 2-4.

- The number of days of hospitalization due to a COPD exacerbation will be recorded during visits 1-4.

- The CRF will be used for this purpose.

- The adverse events occurred during the trial will be recorded during visits 1-4. The CRF will be used for this purpose.

- Any unscheduled visit required due to COPD exacerbations will be recorded during visits 1-4.

- The CRF and CRF of unscheduled visits will be used for this purpose.

- The patients who dropped out from the trial during its course and the cause for the dropout will be recorded during visits 1-4. The CRF will be used for this purpose.

- Any observation deemed appropriate about any aspect of the trial will be recorded in the CRF during all visits.

- The trial monitor will review the CRF during all visits.

9. SAFETY ASSESSMENT

9.1. Specification of Safety Parameters

All adverse events occurring during the course of the trial shall be recorded and assessed in order to evaluate the safety. They will be analyzed thoroughly, both during scheduled controls and whenever a subject notifies an abnormal incident. Exacerbations of COPD will not be considered Adverse Events because they constitute events that are to be expected within the course of the disease and their quantification is the primary objective of this trial.

Adverse events spontaneously manifested by a subject throughout the entire trial should also be recorded, always avoiding questions that might guide the subject's response.

Severity Assessment Definitions:

Mild: Transient symptoms that do not affect the subject's daily activities.

Moderate: Evident symptoms that have a moderate impact on the subject's daily activities.

Severe: Symptoms that have a considerable impact on the subject's daily activities.

All adverse events, both severe and non-severe, will be recorded in the corresponding section of the case report form.

Causal Relationship with the Investigational Medicinal Product

Probable: There are sufficient reasons and documentation to assume the existence of a causal relationship.

Possible: A causal relationship is conceivable and cannot be ruled out.

Unlikely: The event is most likely related to an etiology other than the investigational medicinal product.

9.2. Methods and Schedule Used to Assess, Record, and Analyze the Safety Parameters

Definitions:

- **Adverse Event (AE):** any untoward health occurrence in a Subject or a clinical trial subject treated with a medication and which does not necessarily have a causal relationship with the treatment. Exacerbations of COPD will not be considered Adverse Events because they constitute events that are to be expected within the course of the disease and their quantification is the primary objective of this trial.

- **Adverse Reaction (AR)**: any unintended and harmful response to an investigational medicinal product, regardless of the dose administered.

- **Serious Adverse Event (SAE):**

  Any adverse event that, at any dose:

  - Leads to death.
  - Is life-threatening for the subject (term "life-threatening" included in the definition of SAE refers to the fact that the Subject is at a risk of dying as a result of the event but not that, had the event been more intense, it could have caused the Subject's death).
  - Results in the hospitalization or prolongation of an existing hospitalization.
  - Causes a permanent or significant disability or incapacity.
  - Results in a congenital anomaly or birth defect.
  - Involves the transmission of any infectious agent (through the medicinal product).

  The investigator shall rely on their own medical judgment to determine whether an Adverse Event is serious in other situations. Major medical events that, despite not posing an immediate risk of death or not resulting in death or hospitalization, may endanger the subject or require intervention to prevent one of the other outcomes listed in the above definition, should also be considered serious.

- **Suspected Unexpected Serious Adverse Reaction (SUSAR):** an adverse reaction whose nature or seriousness is not consistent with the product information (e.g., the investigator's brochure in the case of an investigational medicinal product not authorized for marketing or a summary of product characteristics in the case of an authorized medicinal product).

Adverse reactions can be classified into:

- **Local reactions**

  Those appearing in the administration site. They may manifest as a mild burning, swelling, or itchy sensation in the oropharyngeal region. Gastrointestinal discomfort may develop in some cases. The onset of local reactions does not imply that the dosing schedule should be discontinued or changed. Their presence should be carefully assessed.

- **Systemic reactions**

  Those appearing in parts of the body other than the administration site. The severity of these adverse reactions shall be scored according to the following grading system[149]:

  Grade 1: Transient, mild systemic reaction or mild discomfort (<48 hours) not requiring medical intervention nor therapy.

  Grade 2: Moderate systemic reaction leading to a moderate limitation in the subject's activity and requiring barely any medical intervention or therapy.

  Grade 3: Severe systemic reaction leading to a severe limitation in the subject's activity and requiring some sort of medical therapy/care, and, potentially, hospitalization.

  Grade 4: Life-threatening reaction leading to an extreme limitation in the subject's activity and requiring medical therapy and intervention and, probably, hospitalization or palliative care.

Adverse events, broken down by severity and intensity, will be summarized using the MedDRA dictionary, by treatment group, system organ class, and preferred term, indicating the number of subjects per treatment group, the number and frequency of subjects reporting the event, and the number of events.

9.3. Procedures for Recording and Reporting Adverse Events and Intercurrent Diseases, and for Submitting their Reports

All events meeting the definition of an adverse event should be collected and recorded between the start of the first trial-related activity (after the subject's signature of the informed consent) and the end of the post-treatment follow-up period required by the protocol. Subjects will be questioned whether they noticed any adverse event during each visit to the site. All adverse events, whether observed by the investigator or reported by a subject, should be recorded in the corresponding section of the case report form and evaluated by the investigator.

The minimum information to be specified shall be the description, severity, duration, temporal sequence, and method of detection of the adverse event. The treatment administered to manage the event must also be specified.

The investigator shall immediately report all serious adverse events and/or suspected unexpected serious adverse events (SUSARs), regardless of their relation to the investigational medicinal product, by telephone ([redacted] or fax [redacted], to the staff of Inmunotek's Medical Department (always within the first 24 hours of their onset). The initial and follow-up reports shall identify the trial subjects by a unique identification code assigned to each one of them.

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The regulations in force will be followed when issuing the safety information notification to the Health Authorities, the Research Ethics Committees, and the investigators.

If a suspected unexpected serious adverse reaction occurs within the context of a double-blind clinical trial, the treatment code of the particular subject must be disclosed for reporting purposes. Whenever possible, blinding should be maintained for the investigator and the persons responsible for the analysis and interpretation of the results, as well as for drafting the study conclusions.

9.4. Type and Duration of the Follow-up of Subjects After an Adverse Event

9.4.1. Follow-Up of Non-Serious AEs

All non-serious adverse events of severe intensity and possibly or probably related to the investigational product should be followed until the subject has recovered and all concerns have been resolved.

Cases of chronic conditions:

No follow-up is required until the “recovered” status is reached. Once the subject has completed the trial, these cases may be closed with a result category of “recovering” or “not recovered”.

All other non-serious AEs must be followed until the outcome of the event is “recovering” (for chronic conditions) or “recovered”, or until the end of the post-treatment follow-up period set out in the protocol, whichever comes first, and until all concerns related to the AEs have been resolved.

9.4.2. Follow-Up of Serious AEs

All adverse events classified as serious (SAEs) must be followed until the outcome of the event is "recovered", "recovered with sequelae", or "death", and until all concerns have been resolved.

The investigator must send the SAE follow-up information to Inmunotek, S.L., as the trial sponsor, within 5 days of obtaining the follow-up information.

10. STATISTICS

10.1. Description of the Statistical Methods to be Used, Including the Schedule of any Planned Interim Analysis

The study analysis will be performed after the database lock. The intention-to-treat (ITT) population will be composed of subjects who took at least one dose of the trial medication. The analysis will be performed on both the ITT and per-protocol populations.

The randomization will be evaluated by comparing the baseline variables (month 0) in both study groups according to the Consolidated Standards of Reporting Trials (CONSORT) due to their clinical relevance. Qualitative variables will be reported together with their frequency distribution, and quantitative variables will be reported together with their mean and standard deviation, or, in case of asymmetry, with their median and interquartile range. If confounding is detected, the effects will be adjusted with the potentially confounding variables using the appropriate model adjustments.

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 (page 67), 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 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. 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.

To compare the change in the immunological variables described in the trial design (page 67) 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.

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.

The statistical software that will be used is STATA 11.

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10.2. Number of Anticipated Subjects and Rationale. Calculation Method Used for Determining the Sample Size and Data Used for Such Purpose

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.

10.3. Significance Level to be Used

The significance level for the efficacy assessment will be 0.05 for a type I error (α) and 0.2 for a type II error (β).

10.4. Trial Termination Criteria

The trial shall be considered completed when the last recruited subject completes the last trial visit.

10.5. Procedures to Justify Missing, Unusual, or False Data

Prior to the inclusion of the first patient in the trial, the trial monitor will carry out an initiation visit to the site. During this visit, trial investigators will receive training on Good Clinical Practice standards, procedures specific to the trial protocol, and the proper and correct completion of the CRF.

The first trial monitoring visit is planned to take place as soon as the first patient has been included in the trial. The monitor assigned by Inmunotek, S.L. will regularly visit the site during the course of the clinical trial. Each and every one of the trial CRFs are expected to be monitored in order to prevent data loss, identify unusual data, and detect the presence of false data.

In the event of missing data, these will be corrected during the database lock with the last observation carried forward (LOCF) technique, which involves carrying over the last available data to maintain the ITT.

10.6. Procedures for Reporting any Deviation from the Original Statistical Plan

The regulations in force will be followed when reporting information on any deviation from the original statistical plan.

The final report shall include a description of the deviations from the statistical plan that occurred during the course of the trial.

10.7. Selection of Subjects to be Included in the Analysis

Recruited subjects: subjects signing the informed consent, meeting the selection criteria, and assigned a treatment number.

Safety analysis population: randomized subjects who have taken at least one dose.

Per-protocol efficacy analysis population: 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. More detailed information can be found in the Statistical Analysis Plan (SAP).

Intention-to-treat analysis population: all randomized subjects who have taken at least one dose and have been subjected to the week 25 assessment. These subjects will be assigned to the corresponding treatment group.

The medical history and demographic data will be described in the intention-to-treat population per treatment group.

11. DIRECT ACCESS TO THE SOURCE DATA/DOCUMENTS

Source documents are defined as all original documents, data, and records (e.g., medical records, clinical and administrative charts, laboratory reports, subject diaries or assessment questionnaires, pharmacy dispensing records, data recorded by computerized tools, copies or transcriptions certified after being verified as exact copies, x-rays, subject files, and records stored at the pharmacy, laboratories, and the medico-technical departments involved in the clinical trial).

Both the principal investigator and the associate investigators will allow the trial monitors to inspect the source documents and case report forms when requested throughout the course of the trial.

The investigator must also enable access to the source data/documents in the following cases:

- In the event that Inmunotek wishes to perform an audit of the Clinical Trial.

- In the event that the Competent Authorities wish to perform a Clinical Trial Inspection as part of the process of confirming the validity of the trial conduct and the integrity of the collected data.

- In the event that the CRECs involved wish to check the Trial's progress.

During the process of obtaining the subject's informed consent, the principal investigator or associate investigators will request the subject's consent in writing to have direct access to their data. The subject's acceptance to participate in the trial also grants their permission to examine, analyze, verify, and reproduce any records and medical reports that may be relevant for the trial's assessment. Any party (e.g., national and international Regulatory Authorities, trial monitors, and auditors) granted direct access to this information must take all reasonable precautions within the limits of the applicable legal requirements to maintain the confidentiality of the subjects' identities and of the information patented by the sponsor.

12. QUALITY CONTROL AND ASSURANCE

Inmunotek, as the trial sponsor, is responsible for implementing and maintaining the trial's quality assurance and control systems through updated Standard Operating Procedures and the correct monitoring of the Clinical Trial.

To ensure the trial's quality, Inmunotek, through the trial monitor, will verify that:

- The source documents and other trial records are accurate, complete, updated, and correctly filed.

- The data required by the protocol are accurately reported in the CRFs and are consistent with the source data.
- That both the principal investigator and the associate investigators follow the approved protocol and amendments, if any.
- That the written informed consent was obtained prior to each subject's participation in the trial.
- That the investigator and trial staff are performing the specified trial functions as set out in the protocol and have not delegated these functions to unauthorized individuals.
- That the investigator is only recruiting subjects who meet the selection criteria.
- That all doses and/or any treatment changes are well documented for each of the trial subjects.
- That the adverse events, concomitant treatments, and intercurrent illnesses are being reported in the CRFs in accordance with the provisions of the protocol.
- That the visits, tests, and examinations that the subjects failed to complete are clearly reported as such in the CRFs.
- That all withdrawals and dropouts of the subjects included in the trial are reported and justified in the CRFs.
- That all adverse events have been reported appropriately within the time frames established by the GCP guidelines, the protocol, the CREC, the sponsor, and the applicable legal regulations.
- That the investigator is retaining all essential documents.
- That the investigator has been informed of any deviation from the protocol, the Standard Operating Procedures, the Good Clinical Practices guidelines, and the

applicable regulations, and that the necessary measures have been applied to prevent recurrences of the deviations detected.

13. ETHICAL ASPECTS RELATED TO THE TRIAL

All parties involved in this trial undertake to strictly observe the Good Clinical Practice guidelines and the ethical considerations set forth by the current Spanish Legislation governing clinical trials, as well as the corresponding recommendations derived from the European Legislation, as specified in point 3.7 of this protocol.

To ensure the subjects' rights, the Principal Investigator or the associate investigators will explain the trial's objectives and requirements, the nature of the medicinal product under study, and its possible side effects, in a language understandable to the Subject, in the Subject information sheet. The information to be provided includes: a description of the trial objectives, the methodology to be used, the type of treatment, the benefits that the Subject may obtain from the treatment, the risks to which they may be exposed, and the right to withdraw from the trial if they wish to do so.

All subjects must provide their written informed consent before being included in the trial. The informed consent forms shall be stored by the investigator in the trial file during the course of the trial and thereafter for the time stipulated by the legislation in force (at least 15 years).

The research staff will ensure that the relevant consent form is completed according to the subject's age.

14. DATA PROCESSING AND FILING

Inmunotek, S.L. will provide the CRFs that must be fully completed and legible. Any omission of data must be justified in all cases. The member of the research team responsible for making corrections in the cited forms must write their initials and the date on which the correction was made in the location of the amendment.

The investigator shall submit a list with the signature and handwritten initials of each of the collaborators authorized to make data entries or changes in the case report forms. If any modification is made throughout the course of the study, an updated list of authorized signatures must be provided.

Subject data recorded in the CRFs during the course of the trial must be documented in an anonymous manner, exclusively identifying the subject by their assigned number. If the subject needs to be identified due to exceptional safety reasons or by indication of the Regulatory Authorities, the investigator will be obliged to maintain the confidentiality of such information.

The investigator must retain the original documents for each trial subject comprising all demographic and medical information, including laboratory data, and keep a copy of the signed informed consent form. All information recorded in the CRFs must be traceable to the original documents stored in the subject's file. Data without an original written or electronic record will be defined before the start of the trial and directly recorded in the CRFs, where they will be documented as original data.

The investigator must safeguard and maintain the essential documents defined by the ICH guidelines, the Good Clinical Practice guidelines, and the local regulations for at least 15 years following the trial's termination or completion. The Subjects' medical records and other original data will be retained for the maximum time permitted by the hospital. To this end, the sponsor shall provide the investigator with a file, which the investigator must update as often as necessary.

The investigator's file must be accessible during monitoring visits, and in case of an audit and/or inspection.

Inmunotek, S.L. is responsible for the updated master file of all documentation relating to the trial and must keep all this information during the entire shelf life of the medicinal product. Any change in the data ownership must be documented. In all cases, the documents must be made available to the competent authorities if so requested by them. Once the trial period has ended for each subject, the trial monitor will collect their case report form, which they will subsequently review, recording any

discrepancy, concern, or comment about it in a separate document. The monitor shall discuss this list of discrepancies with the investigator in order to correct the corresponding data in the case report form. Any corrections resulting from this discussion must be made by the authorized research staff. Neither the trial monitor nor any delegate of Inmunotek, S.L. is authorized to enter data or make corrections in the case report forms.

Upon completion of this review, the data corresponding to all the subjects included in the trial will be computerized, using a double data entry system, for their subsequent statistical analysis. Once processed, the original forms will be filed together with all relevant trial documentation in the Medical Department of Inmunotek, S.L. This file will be clearly identified in case its review or audit is required.

15. FINANCING AND INSURANCE

Inmunotek, S.L. has taken out a Civil Liability insurance policy in accordance with the current legislation.

16. PUBLICATION POLICY

The results or conclusions derived from this trial shall preferably be disclosed in scientific publications referencing the corresponding Ethics Committee. The anonymity of the subjects participating in the trial shall be maintained at all times.

The partial or full publication or disclosure of the data or conclusions obtained from the clinical trial will be performed by mutual agreement between the investigator and Inmunotek, S.L. If the Trial Sponsor fails to do so within one year, the investigators will be free to publish the results obtained in the trial.

17. ANNEXES

N/A

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Witness my hand, this 02nd day of June 2021

Signed: [redacted]

[redacted]

Don [redacted] Traductor-Intérprete Jurado de lengua inglesa, nombrado por el Ministerio de Asuntos Exteriores y de Cooperación, certifica que la que antecede es traducción fiel y completa al inglés de un documento redactado en lengua española.

En Getafe, a 02 de junio de 2021

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