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Introduction of BCG vaccine as immunotherapy on pulmonary disease due to *Mycobacterium Avium Complex*:

Effectiveness determination (II phase)

A multicentre, superiority, randomized, double-blinded and controlled clinical trial

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Al meu àngel de la guarda, sempre ets aquí.

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"En Ell heu rebut tota mena de riqueses, tant de paraula com de coneixement."

1Cor, 1:5

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1. ABSTRACT

Background

Nontuberculous mycobacterium (NTM) infections are increasingly frequent due to the human engineering environment, the susceptibility of the hosts, and the improvement of diagnostic techniques to detect and typify them. The most common pathogenic specie is the *Mycobacterium Avium complex*, which usually causes chronic lung disease in immunocompetent patients. The current antimicrobial treatment is long (12-18 months), with high toxicity (due to the use of antituberculosis drugs), low cure taxa (50-55%) and high reinfection taxa (up to 40%).

Recent studies have demonstrated the existence of cross-immunity between TBC and MAC from the application of BCG vaccines in multiple murine models, nonhuman primates and human subjects. These studies have revealed an inhibition in the replication and growth of MAC-infected cells, opening a gap to induce the treatment of these patients with BCG immunotherapy.

Objective

The purpose of the present study is to demonstrate the effectiveness of BCG vaccine against tuberculosis as induction immunotherapy for the treatment of pulmonary infections due to Mycobacterium Avium Complex.

Methods

Design and setting: A multicentre, superiority, randomized, double-blinded and controlled clinical trial will be performed among eight hospitals of Catalonia.

Intervention: A stratified by radiological pattern sample will be performed in order to generate two therapy groups (A and B) with participants >18 years old. Patients from **group A** (n = 108) will receive intradermal placebo (two dose) followed by oral antibiotic until microbiological healing and 12 months since that moment while **group B** patients will be stratified in two groups that will receive two different dose of BCG vaccine followed by the same antibiotic guideline, **group B-I** (n = 108) and **group B-II** (n = 108).

Treatment will last maximum 18 months, depending on the time it takes for the patient to reach microbiological healing (rarely happens after 6 months).

Main outcome will be disease resolution, defined as the negativization of sputum culture and maintained disappearance of symptoms for at least twelve months since it occurs. One follow-up visit per month (4 weeks) will be scheduled after diagnosis for both groups, and will consist mainly in clinical examination of symptoms and monitoring of hepatic function, EKG and sputum culture.

Keywords: *M. Avium Complex*, pulmonary infection, immunotherapy, BCG, disease resolution.

2. ABBREVIATIONS

NTM – Nontuberculous mycobacterium

AIDS – Acquired immunodeficiency syndrome

TBC – Tuberculosis

COPD - Chronic obstructive pulmonary disease

ATS/IDSA – American Thoracic Society / Infectious Diseases Society of America

HAART – highly active antiretroviral treatment

IRIS – immune reconstitution inflammatory syndrome

MAC – Mycobacterium Avium Complex

MAV – Mycobacterium Avium

MAB – Mycobacterium Abscessus

PID – pulmonary interstitial disease

HRCT - high resolution computerized tomography

DST – Drug susceptibility test

CLSI – clinical & laboratory standard institute

HUDJT – Hospital universitari Doctor Josep Trueta

CEIC – Clinical Research Ethics Committee

AEMPS – Asociación Española del Medicamento y Productos Similares

3. INTRODUCTION

NTM general aspects

Unlike the main pathogenic mycobacteria groups (i.e. *M. leprae* and *M. Tuberculosis*), nontuberculous mycobacteria (NTM) are, in most cases, opportunistic human pathogens that normally inhabit in the environment. We currently know over 150 individual species, but most of them are relatively new appeared, owing to the increasing susceptibility of hosts and the improved techniques for culture and detection (1).

They behave as environmental pollutants in swamp soils and water, especially in backwaters of the human engineered environment as domestic network of water, hot tubs, swimming pool, homes and workplaces (including hospitals for their resistance to chlorine and other disinfectants, with cases of transmission through contaminated bronchoscopes) (2). Once they are introduced, they can attach to surfaces and grow to form a protector biofilm, that improves its resistance and makes easy to become a source of infection (1).

As opportunistic pathogens, they require an alteration of the host defending mechanisms. Because of this, they are in association with alterations of the mucous barrier, underlying lung disease or immunocompromised states (2).

Pathogeny

NTM physiologic characteristics are the most important determinants of their occupancy.

1. They have a hydrophobic lipid-rich membrane that provides impermeability to hydrophilic compounds such as disinfectants and antibiotics.
2. Its slow growth is not due to a slower metabolism, but the fact that a substantial amount of energy and carbon are diverted to the synthesis of the outer membrane. This coupled to a rapid metabolic rate, allows NTM to adapt in changing and hostile conditions.
3. NTMs can metabolize complex organic compounds that other drinking water microorganisms cannot.
4. Finally, they have good resistance to heat (approximately 50-60ºC) and preference for growth at acidic pH (1).

Transmission

Different transmission pathways for NTMs have been proposed. Among the most common we can find aerosolization and inhalation (in lung disease), swallowing and aspiration and introduction into wound from water and other materials (either injury or surgical intervention) (1). There are no many cases of transmission between patients because NTMs are environmental. Also digestive pathway in case of AIDS and children (3). **Figure 1** summarises the functioning of the immune response that triggers the infection.

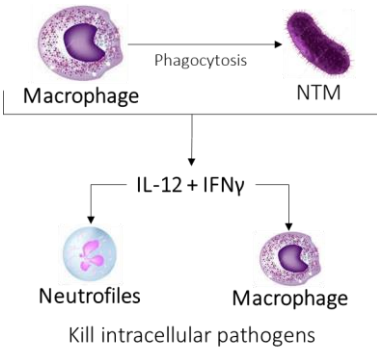

Figure 1. *Immune response triggered by the infection*

Epidemiology

Because of its environmental sources and pathogeny, NTM disease is more frequently found in patients of developed countries and rarely in patients from developing countries. The opposite happens with TBC, with higher prevalence rates in developing countries than in developed countries (4).

In Spain, infections produced by NTM are not mandatories of systematic reporting (4), making incidence and prevalence data approximate, closely linked to the possibilities of isolation and identification of local laboratories (3).

The **most recent study of Catalonia** was conducted with data from three hospitals in the southern health area, and collected data included in a period of 21 years (1994-2014). The prevalence of patients from whom NTM was isolated was 113.2 / 100,000 population; for pulmonary disease, prevalence was 42.8 / 100,000 population (4).

M. Kansasii was the most frequently isolated NTM in Catalonia during the 90ies due to HIV epidemic in Spain. That was frequently isolated in patients with AIDS until the introduction of antiretroviral treatment.

MAC and *M. Abscessus* are more frequently isolated in patients with underlying pulmonary disease. The increased susceptibility of population to suffer COPD, asthma and bronchiectasis, for example, made this mycobacterium the most frequent currently **(Figure 2)** (4).

Authors also found that trends in prevalence rates differed among spices of mycobacteria and that in some there was a significant difference in some cases about the colonization and disease(4). *M. Avium* is suggested as the main source of infection in all patients. *M. Kansasii* is the second most frequently isolated NTM in patients without immunosuppression (3).

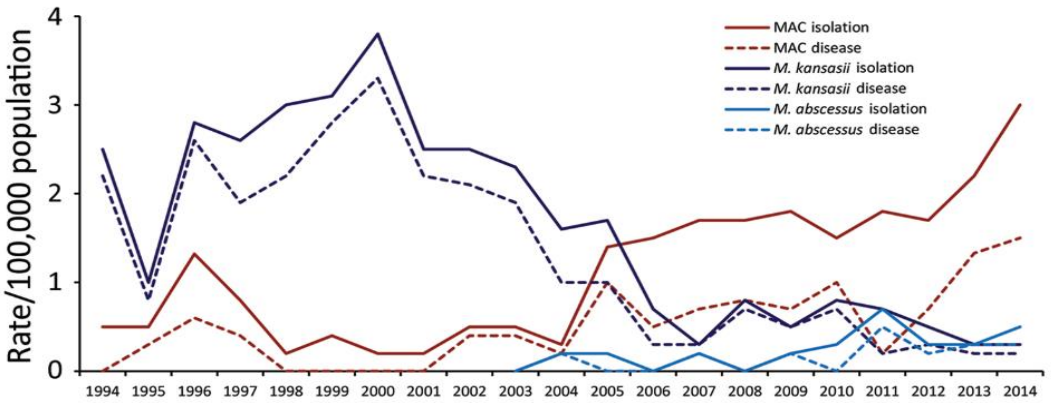

Figure 2. Annual prevalence rates per 100,000 population of 3 species of nontuberculous mycobacteria (Mycobacterium kansasii, MAC, and M. abscessus), Barcelona-South Health Region, Catalonia, Spain, 1994–2014 (4) . MAC: Mycobacterium avium complex

Girona's population: A recent study about epidemiology performed HUDJT (Girona) describes that in the last 9 years, it has been isolated 162 NTM of 15 different species. *M intracellulare* and *M. Avium* were the most frequent (61%), followed by *M. Abscessus* (14.8%). Other isolated species were also *M. Fortuitum, M. Gordonaem M. Bovis, M. Xenopi* and *M. Inmunogenum* among others less frequent. This sample was mostly composed by males (67%) with mean age of 63. Finally, they observed a growing progression of NTM isolates throughout the study period in relation to a slight decrease in cases of TB treated in the same centre (51).

The diagnostic criteria established by the document of the America Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) **(Table 1)** are those that have marked, since 2007, the management guidelines for those patients, and are still used nowadays (5).

Decrease of TBC, mainly in industrialized countries, has been confirmed since the correct application of this criteria to diagnose NTM infections, that have increased progressively the incidence of pulmonary form (2).

The majority of epidemiological studies carried out in recent years that have measured fluctuations in the incidence of *M. tuberculosis* and NTM infections have concluded that, there is a significant relationship between the increase in NTM prevalence and the decrease in prevalence of TBC (4,6–12).

***Table 1**. Clinical and microbiologic criteria for diagnosing NTM lung disease (ATS 2007 criteria)*
CT: computerized tomography; NTM: nontuberculous mycobacterium; AFB: acid fast-bacilli

Clinical (both required)	1. Pulmonary symptoms, nodular or cavity opacities on chest radiograph, or high-resolution CT that shows multifocal bronchiectasis with multiple small nodules 2. Appropriate exclusion of other diagnoses.
Microbiologic	1. Positive culture result from at least two separate expectorated sputum samples. If the results from (1) are nondiagnostic, consider repeat sputum AFB smears and cultures. or 2. Positive culture result from at least one bronchial wash or lavage. or 3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM. 4. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination. 5. Patients who are suspected of having NTM lung disease but do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded. 6. Making the diagnosis of NTM lung disease does not, per se, necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients.

Risk factors

Initially, when NTMs were isolated in a respiratory sample they were considered as contaminants. They were described as infectious pulmonary disease occasionally in immunocompromised patients and those who had underlying pulmonary disease (2).

Nowadays we know that the most frequently risky factors associated to this disease are smoking and underlying pulmonary disease such a pulmonary disease chronic obstructive pulmonary disease (COPD) or silicosis and bronchiectasis(3).

The significant increase in incidence may be related to different factors, especially the increasing of COPD, the improvement of diagnosis techniques and clinical recognition, and its description in immunocompromised patients (particularly AIDS epidemic, but also neoplasms, immunosuppressive treatments, transplants, etc.). It has been described even in children (3).

Role of underlying lung disease:

Since the first isolations, the debate continues as to whether the isolation of NTM in these patients is a contaminant, coloniser or pathogen. Indeed, the pathogen may have a very slow burning course (difficult to detect in these patients) or an aggressive course which can be detrimental.

Overtime, it became clear that underlying lung conditions such as **TBC, pneumoconiosis, cystic fibrosis** (increases with the age), **bronchiectasis** (as risk factor or as sequel), **pulmonary alveolar proteinosis** and **silicosis** were all associated with NTM infection. Chest wall deformities as *pectus excavatum* and scoliosis are related with *M. Avium* (8).

Role of immunosuppression:

HIV positive patients are usually predisposed to disseminated disease once the CD4+ count is <50/μl. Disseminated disease may manifest itself with lymphadenopathy, hepatosplenomegaly and anaemia as main symptoms. Other symptoms include anorexia, weight loss, night sweats and fever. They rarely have clinically important lung disease (8).

It is difficult to treat the HIV positive patients. Rifampicin increases the clearance of HAART, and this may lead to resistance to this antiretroviral treatment that save their live. HAART may also inhibit the metabolism of rifampicin leading to toxic levels accumulating (13).

NTM infection has also been described in patients who have undergone **solid organ or stem-cell transplantation**. This type of infections affects mainly heart and lung transplantation.

NTM in patients with renal transplantation was studied by Queipo et al. in 2003 (14) with a retrospective analysis of 1261 cases in Spain. They found *M. Kansasii* infection in 5 of them, located in the kidney, and they also described renal transplantations complicated by lung infections (*M. Chelonae and M. Xenopi*) (14). It is also difficult to treat these infections because of the interactions between antibiotics required and immunosuppressive drugs (8).

Pulmonary disease due to *Mycobacterium Avium Complex*

Table 2. NTM causing lung infectious disease in humans (15)

Slow growth NTM	Rapid growth NTM
M. Avium complex	***M. Abscessus***
M. Avium	*M. Chelonae*
M. Intracellulare	*M. Fortuitum* group
M. Kansasii	*M. Fortuitum*
M. Malmoense	*M. Peregrinum*
M. Xenopi	*M. Fortutium biovar*
M. Szulgai	*M. Mucogenicum*
M. Scrofulaceum	*M. Immunogenum*
M. Haemophilum	*M. Smegmatis*
M. Simiae	*M. Wolinskyi*
M. Celatum	*M. Goodii*
Complex *M. Terrae*	
M. Gordonae	

Species that form colonies on culture in 7 days or fewer are termed *"rapidly growing mycobacteria"* while species that require more than 7 days are classified as "*slowly growing mycobacteria"* (Table 2).

Depending on the species, some slowly growing mycobacteria can take 4 or more weeks to form mature colonies (16). All of them can cause very different clinical forms, like *M. kansasii, M. Avium Complex* or *M. Abscessus*, summarised in Figure 3.

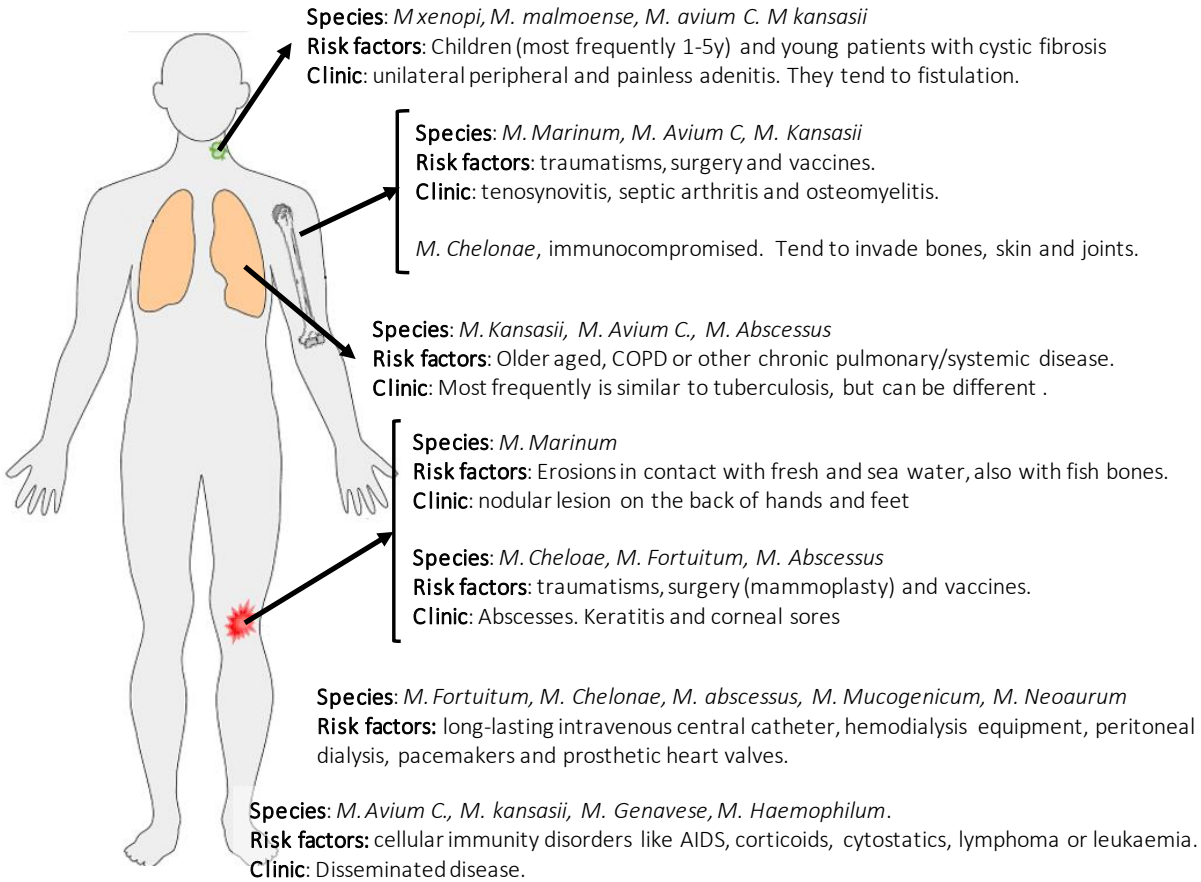

Figure 3. Different clinical forms produced by different species of NTM

MAC clinical forms

Mycobacterium avium complex is a slow growing Mycobacterium that is adapted to grow at 37°C. In Löwenstein-Jensen (LJ) culture, initially makes small and non-pigmented smooth colonies, including:

- *M. avium*: avium subspecies, *Colombian*, *silvaticum*, *hominissuis* and *paratuberculosis*.
- *Mycobacterium Intracellulare*
- *Mycobacterium chimaera*

It is associated to different serotypes with epidemiological, pathogenic and clinical differences.

- *M. Avium* serotypes 1 to 6, 8 to 11 and 21, is more frequent in HIV positive patients, with systemic involvement.
- *M. Intracellulare* serotypes 7, 12 to 20 and 22 to 28 is more isolated in patients with pulmonary interstitial disease (PID), that presents two different computerized tomography (CT) patterns: apical fibrocavitary disease or nodular bronchiectasis pattern.

Since the AIDS pandemic, MAC becomes the main NTM associated with disseminated infection and/or lung involvement, gastrointestinal tract or lymphatic involvement. Its incidence has decreased significantly in HIV patients due to the introduction of antiretrovirals. MAC has also been associated with Lady Windermere syndrome or hypersensitizing pneumonitis.

MAC is distributed worldwide, although its presence is more prominent in temperate regions of the United States, Europe, Japan and South Africa (16). In most geographical regions, *M. Avium Complex* is the main causal agent of pulmonary disease, leading to morbidity and mortality, especially in patients with PID but also in previously healthy patients.

At the beginning, *M. Avium* and *M. Intercellulare* were different classified because of its pathogenies in poultry and rabbits respectively. Nowadays, is considered that clinical and radiological manifestations and the response to antimicrobial therapy are practically indistinguishable (17).

Pulmonary disease

Pulmonary disease is the most common clinical form caused by NTM, especially due to *M. Avium Complex* (MAC) and *M. Abscessus*. In this case, patient's isolation is not necessary (difference with TBC) because it has not been described the transmission between persons.

The type of pulmonary disease is conditioned by the patient's immune status and the specie of NTM (15). Nonetheless, the two main radiological manifestations are the 1) apical fibrocavitary disease, and the 2) nodules and bronchiectasis

- Apical fibrocavitary disease (Figure5), commonly present in men, middle-aged or elderly smokers with chronic obstructive pulmonary disease, which clinically and radiologically similar to tuberculosis. This form of presentation can also occur in people without predisposing factors.
- Nodules and bronchiectasis, more frequent in women >50 years, immunocompetent, and without smocking habit nor previous lung disease. It is difficult to diagnose, and it has a progressive course.

The infection can be developed on bronchiectatic areas, habitual in patients with previous TBC that present new radiologic infiltrations or in cystic fibrosis (18).

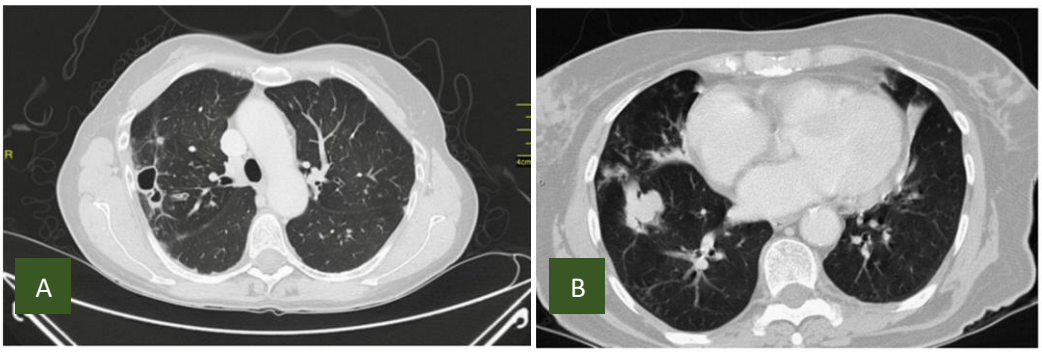

***Figure 4**. Comparison between fibrocavitary (A) form and nodular-bronchiectatic form (B)* (22)

Regarding the symptoms, almost always is very similar to TBC lung infection: chronic and progressive symptoms and tendency to cavitate. Presentation is unspecific, with cough, asthenia, weight loss, fever, dyspnoea and occasional haemoptysis (15). Despite this, we can also find specific syndromes:

- Lady Windermere Syndrome (Figure 6): occurs in women over 50 years, with a thin constitution and without predisposing diseases, although they frequently present with thoracic abnormalities (i.e. *pectus excavatum,* scoliosis and mitral valve prolapse) and Marfanoid habit. Pulmonary lesions are usually of the nodular or interstitial type, with bronchiectasis preferentially located in the middle lobe and lingula (15,18).
- Hot tub lung syndrome: presented as hypersensitivity pneumonitis, related to bathtub water. It is not clear if it is infectious, immunologic, or both.

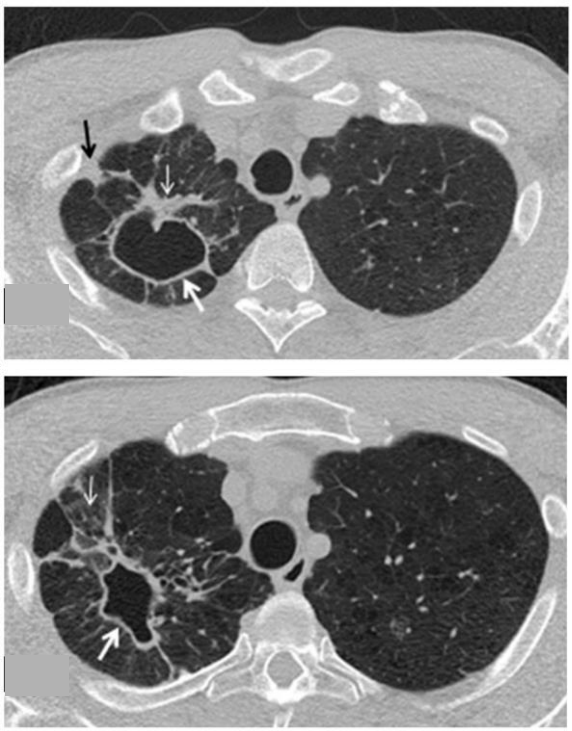

Figure 5. *Fibrocavitary "tuberculosis like" form* (64)*: Patient of 65 years, hard smoking that presented cough, dyspnoea and 11 months loss of weight. Bacilloscopy was negative and bronchial lavage showed MAC.*

This HRCT demonstrates the presence of a cavity an apical cavity of thin walls surrounded by areas of consolidation, areas of density in frosted glass and pleural thickening.

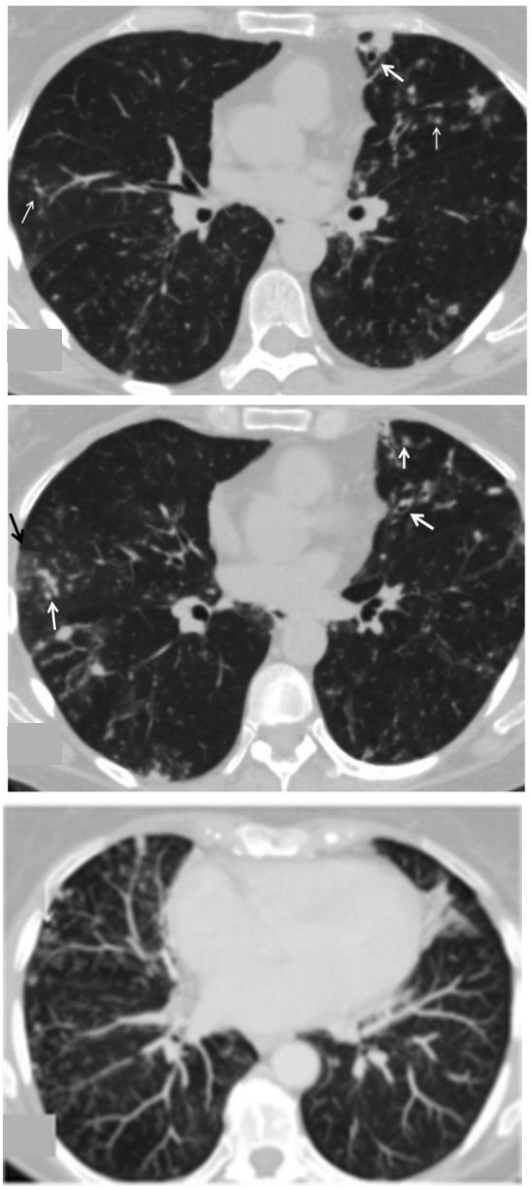

Figure 6. *Lady Windermère Syndrome* (64)*: Patient of 63 years that presented cough, dyspnoea and 8 months loss of weight. Bacilloscopy was negative and bronchial lavage showed MAC. She realized different antibiotic guidelines that had unfavourable results*

This CT shows presence of micronodules in centre of lobule, bronchiectasis and isolated areas of frosted glass density. Affectation is mainly located in medium lobule.

In last image, note tree-in-bud pattern

Adenitis

Adenitis is the most common mycobacteriosis in children <5 years old. Nowadays, the most frequent specie that most frequently causes it is MAC. Clinical manifestation consists of a painless adenopathy (1-6cm diameter; only one in most cases) located on the submandibular, cervical anterior or preauricular region. It fistulizes from 3 weeks to months.

Musculoskeletal infection

Although *M. Marinum* is the most isolated microorganism in musculoskeletal cases, MAC can cause tenosynovitis, frequently due to traumatisms, vaccines or surgery.

Disseminated disease

Disseminated disease appears frequently in patients with immunosuppression due to some diseases like AIDS, hematologic neoplasia, long-term treatment with glucocorticoids or after transplantation (15).

Diagnosis

Establishing the definitive diagnosis is as important as difficult, since subsequent treatment will depend on it. The main challenge is to find the species that causes the disease and typify it in order to establish an effective treatment.

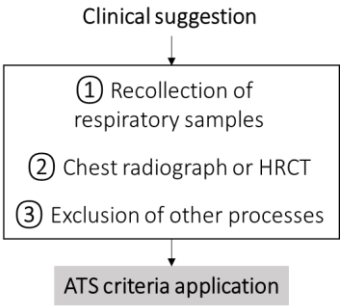

Figure 7. Diagnosis algorithm
HRCT: High resolution CT

Aforementioned ATS criteria fit best with MAC. The minimum evaluation of a patient suspected of NTM lung disease should include **(Figure 7):**

1. **≥3 sputum specimens for their analysis**. Sample must be recollected in morning and on an empty stomach. The three sputum must be 5-10mL in 3 consecutive days. If collecting spontaneous sputum is not possible, it can be induced with mechanical manoeuvres as "clapping" or (although not usual) by bronchoscopy technic. In this case, is necessary more than 5mL sample. Regarding respiratory biopsies, a minimum of 1g sample tissue is required in bronchial, transbronchial, pulmonary and pleural samples.
2. **Chest radiograph** or **HRCT scan** in absence of cavitation.
3. **Exclusion of other lung disorders** such as TBC and malignancy (5,19).

Treatment

Pulmonary MAC infections are difficult to treat for two major reasons. First, the treatment regimens are very long, requiring the use of multiple drugs for at least 18 months. Second, the failure and relapse rates may exceed 40%. Therefore, strategies to improve the prevention and treatment of NTM infections in high risk patients are needed (20).

In order to establish a treatment guideline, we must consider some premises. They are more resistant to anti-TBC drugs than TBC, so therapy must be individualized. The accumulated experience is lower than with TBC (very scarce in some species). There is not necessity of treating colonisation cases if they are not matched with clinical manifestations, therefore in this case we must balance the benefit of the treatment related with toxic effects of drugs and disease progression (treatments based on suspicion are not indicated) (15).

DTS

Currently, uniform agreement concerning the indications for susceptibility testing of isolates of MAC is not clear. However, investigators from CLSI who have extensively studied MAC disease have recommended in 2003, 2011 and 2019 guides that isolates should be tested in the following situations:

- Clinically significant isolates from patients on prior macrolide therapy
- Isolates from patients who develop bacteraemia while on macrolide prophylaxis
- Isolates from patients who relapse while on macrolide therapy
- Initial isolates from blood or tissue (e.g. from patients with disseminated disease) or from clinically significant respiratory samples (e.g. sputum or bronchoalveolar lavage fluid) to establish baseline values.

If baseline testing is not performed, saving the isolate for future testing (if necessary) is strongly recommended.

Susceptibility testing should be repeated after 3 months of treatment for patients with disseminated disease and after 6 months for those with chronic pulmonary disease in case that the patient shows no clinical improvement or clinical deterioration and is still culture positive (16,21).

Antibiotic guidelines

Some studies have evaluated the response to anti-TBC medications and their relapses after medical therapy. Results were not many encouraging. It has been demonstrated that the **negativization of cultures with clinical remission occurs in no more than 50-60% of cases**, and about **40% of them relapse over time** (5,22).

Table 3. Antibiotic treatment guidelines (5,23)

Nodular or bronchiectasis	**Clarithromycin*** 1g or Azithromycin 500mg + **Ethambutol** 25mg/kg + **Rifampicin** 600mg Three times per week
Fibrocavitary or severe	**Clarithromycin*** 500mg-1g or Azithromycin 250mg + **Ethambutol** 15mg/kg + **Rifampicin** 10mg/kg every day. May be considered the addition of Streptomycin or Amikacin (aerosol + systemic 1mg endovenous or intramuscular 3t/w in 2-3 first months)

** If clarithromycin is not an option, it can be replaced by Moxifloxacin 400mg/d or Levofloxacin 500-750mg/d.*

Initial treatment of pulmonary MAC infections must include a combination of macrolides (Clarithromycin or Azithromycin), ethambutol and rifampicin. ATS guides of 2007 recommend rifampicin over rifabutin, as well as differentiating two dose guidelines according to whether it is a nodular-bronchiectasis or multibacillary fibro-cavitary disease (see **Table 3** for an explanation).

In the first one, more severe, recommendation is to administrate a daily dose of oral drugs associated to aminoglycosides (amikacin or streptomycin). For nodular paucibacillary forms is better to administrate drugs three times a week without aminoglycosides (22).

There is a resistance problem with clarithromycin if it is administrated as mono or biotherapy. In case of resistance, there are some alternatives to clarithromycin, as moxifloxacin and linezolid (24).

In case of lymphadenitis, the infected lymphatic can be surgically removed as treatment. If there is no response, or there is resistance to macrolide, a partial resection of lung could be indicated (23).

Monitoring of the response

Goals of therapy include symptomatic, radiographic and microbiologic improvement. Cultures of sputum should be obtained monthly during therapy for pulmonary MAC disease to assess the response. Patients should show clinical improvement within 3 to 6 months and should convert their sputum to negative within 12 months on macrolide standard regimens (25).

Symptomatic improvement is also important, but not always easy because most of these patients can have an exacerbation of their underlying diseases as bronchiectasis and COPD.

Radiographic assessment can be difficult to do because pulmonary abnormalities due to an underlying pulmonary disease can often interfere with the residual forms of infection (5).

As previously mentioned, when sputum cultures are negative, it is indicated to continue treatment for one year before removal. This end-point is well established, as several studies have shown that recurrence after one year of negative cultures is due to reinfections by other MAC serotypes (27).

Furthermore, hepatic function and electric activity of hearth must be monitored every 2 months of treatment through an analysis and EKG due to the high toxicity of antibiotic treatment. We will assess a change in pattern if liver values rise 5 times without symptoms or 3 times with symptoms (23).

Limitations of the current treatment

The biggest challenge in NTM treatment in general is the widespread view that share a similar pathogenesis of TBC, so it is assumed that drug susceptibility testing and treatment are the same in both cases (28).

When specie is isolated, it is difficult to detect the drug resistance profiles that allows to treat patients effectively. NTM drug susceptibility test (DTS) is the same that the one used in TBC and it is not as effective as it is with TBC (28).

Their resistance mechanisms are assorted:

- Antibiotic inactivation: beta-lactamases, aminoglycoside phosphotransaminase and aminoglycoside acetyltransferase.
- NTM p55 flux pump confers resistance to tetracyclines and aminoglycosides.
- Erythromycin ribosomal methylase: located in *M. Abscessus sp*, make them resistant to macrolide (28).

Particularly in MAC infections, there is a problem with DTS and clinical correlation. This is because in most of cases, their real capacity in front of the infection is not reflected on cultures. This capacity is lower when we apply the treatment in the real patient.

Most of times, cultures are resistant to most of antibiotics except macrolides.

Another problem is the great toxicity and treatment side effects. The most frequent and common side effects in antibiotics are hypersensitivity and gastrointestinal disturbance. However, these side effects could be more serious, such as hepatitis or hematologic pancytopenia.

There are other specific side effects, characteristic of some of the drugs we use **(Table 4)**. Interactions with other medications almost never happen (5).

***Table 4**. Adverse events of antibiotics*(29)*, modified*
Flourquin: fluoroquinolones

Macrolides	Gastrointestinal disturbances (diarrhoea, vomiting and nausea, abdominal pain), QT prolongation
Rifampicin	Nausea, rash and occasional hepatotoxicity, flulike syndrome
Rifabutin	Uveitis
Fluoroquin.	Gastrointestinal disturbances, tendonitis, QT prolongation
Ethambutol	Optic neuritis
Linezolid	Neuropathy, thrombocytopenia, myelosuppression and optic neuritis
Isoniazid	Liver, peripheral neuropathy

In addition, patients often suffer from underlying lung disease. This long and aggressive treatment adds comorbidities to their baseline state, taking into account that microbiological healing is only assumed in 50-60% of them and the chances of reinfection are high (22).

Immunity and vaccine

Calmette-Guérin Bacillus

Humans have been infected with *M. tuberculosis* for millennia, characterized by a complex immunologic response that leads to a unique host-pathogen interaction, making it difficult to treat and control.

The introduction of Bacille Calmette-Guérin (BCG) and chemotherapy in the past century marks an important advance in the history of tuberculosis (TBC). This generated some optimism in the fight against the disease especially in endemic area (30).

BCG was **first used in humans in 1921**. It is a live attenuated bacterial vaccine derived from *M. Bovis* that was originally isolated in 1902 from a tuberculous cow. Currently is the only available TBC vaccine.

BCG has demonstrated **significant effectiveness** in several populations, although not consistent against all forms of TBC **(0-80%)** and in all age groups. It is also widely used as treatment against bladder cancer since the eighties (31).

Immunological reaction triggered by the vaccine

Innate

The initial immune response to BCG occurs at place of inoculation (usually the dermal layer of the skin), where resident **macrophages** and **dendritic cells** interact with the bacillus via different receptors that are expressed on their surface.

Macrophages and dendritic cells phagocytose the bacteria initiating the innate immune response through the secretion of immunomodulatory components such as cytokines (**TNF, IL-1, IL-6, IL-10, IL-12**) and chemokines.

Bacteria are degraded via intracellular killing mechanisms. Their peptides are trafficked to the plasma membrane along with **major histocompatibility complex** (MHC) **class I and II,** where they are presented to cells of the adaptive immune system.

Neutrophils also enter the site of inoculation and participate in the response.

Finally, dendritic cells, loaded with bacteria and expressing antigen on their surface, home to draining lymph nodes.

Adaptative:

When they enter inside the **lymph nodes**, dendritic cells stimulate **CD4+, CD8+, CD1+-restricted T cells** (unknown function), **TFH**, **T regulatory** cells, and **B cells**. CD4+ and CD8+T cells migrate out of the lymph nodes toward the site of inoculation and provide the necessary stimulation to innate cells.

CD4+T cells differentiate into Th1 (mediation in part by IFNγ), Th17, or Th2 cells depending on the stimuli present in their microenvironment and aid in the activation of macrophages. **CD8+T** cells mediate their functions by lysing infected cells or by secreting cytokines (robust response of IFNγ production).

B cells differentiate into antibody producing plasma cells (IgG 1, 2 and 3 and IgA to promote phagocytoses) or memory B cells. Throughout the process, memory cells arise from those that responded to the infection and populate peripheral organs, such as the lung.

Together, the cells of the adaptive immune systems orchestrate the immune response in an attempt to establish mycobacteria immunity (32).

This process is summarised in **Figure 8**.

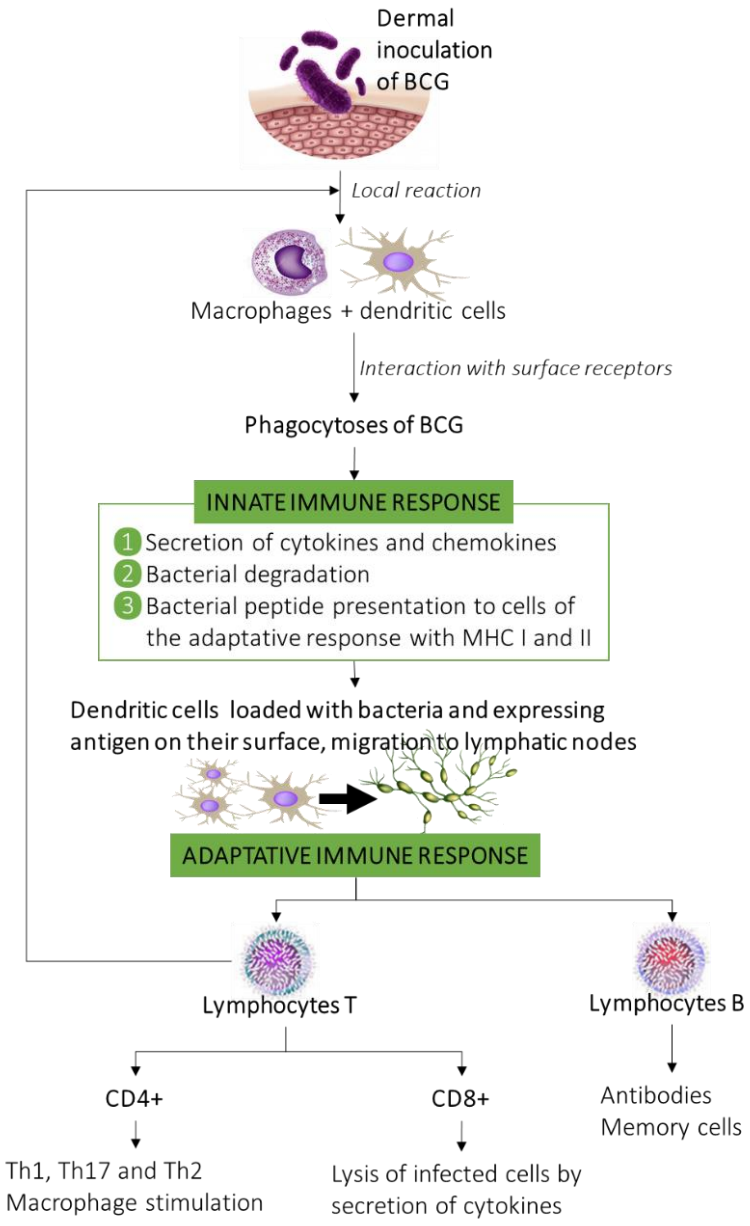

***Figure 8**. Immune response triggered by BCG*

Heterologous mycobacterial immunity

As TBC, MAC is an intracellular pathogen, so cell mediated immunity plays a major role in protection. Therefore, **vaccine strategies for NTM should be similar to strategies employed for TBC,** relying mainly on inducing or boosting protective cell mediated immunity (20).

Notably, there appears to be an overlap between protective immunity for TBC and that of NTM. For instance, epidemiological studies indicate that BCG vaccination is associated with marked decreases in *Mycobacterium avium* (MAC) disease prevalence (11). Similarly, latent TBC infection decreases the risk of NTM disease, further suggesting the importance of **cross-protective immunity**. However, the basis for this cross-protective immunity and cell types involved in cross protection are not known (20).

The mechanisms by which NTM induces innate immune responses are not well understood for BCG or *M. tuberculosis*, but the genus *Mycobacteria* shares many similarities with them, including the complex and lipid-rich cell wall.

There are also differences. NTM persists and replicates within macrophages, but its survival strategies are variable and generally different from *M. tuberculosis*. Moreover, the variability between the forms of induction of autophagy once inside the macrophages is also different from *M. tuberculosis*. Variations in cell death induction capabilities are also available (33,34).

Such variability of exposure to NTM species can differentially influence immune responses to BCG and *M. tuberculosis* (33). In 1958, the Public Health Service and the US Navy instituted a cooperative program designed to study skin-test sensitivity and related tuberculosis morbidity among navy recruits by using PPD of *M. Tuberculosis* and *M. Intracellulare*. In this study, they observed that the incidence of active tuberculosis disease was lower in individuals with a larger *M. Intracellulare* than *M. tuberculosis* in skin test result (35).

Epidemiologic evidence supports the hypothesis that NTM induce protective effects from prior infection with *M. tuberculosis*. NTM rates are increasing worldwide, coincident with a decline in tuberculosis, potentially indicating that tuberculosis disease and infection with *M. tuberculosis* may provide some protection against NTM disease (33).

First investigations with animal models

Early animal models consistently demonstrated that NTM infections protect animals from subsequent challenge with *M. tuberculosis* (36). Adoptively transferred T cells from mice infected with *M. tuberculosis* provided protection from challenge with selected NTM, supporting the concept that cross-protective T cells exist among different *Mycobacteria* species (37).

Studies of the effect of NTM on BCG vaccine responses are difficult to interpret, but in most cases, they suggest that NTM do not interfere with the protective effects of BCG vaccination. Experiments conducted in the 1980s found that prior aerogenic infection with *M. kansasii, M. Simiae, M. avium*, and *M. Scrofulaceum* protected mice from subsequent *M. tuberculosis* challenge and did not alter the capacity for BCG to influence *M. tuberculosis* bacterial burden (38).

Interestingly, this effect was not uniform among all species of NTM; protection was conferred against *M. avium* and *M. kansasii* but not *M. Simiae* aerosol challenge. The behaviour of infection, species of NTM, capacity for NTM to replicate intracellularly, and duration of NTM exposure may play an important role in influencing cross-protection.

Thus, studies carried out under different conditions would have a different response to the BCG vaccine post-infection by NTM that would interfere with the efficacy of that vaccine. Variation in NTM exposure, including dose, species, and strain, influences BCG responses and *M. tuberculosis* heterologous immunity (33).

The route of BCG administration may mitigate the immunologic effects of oral NTM exposure. Price et al (39) demonstrated that oral NTM influenced T-cell responses to intradermal but not inhaled BCG.

Cross – immunity induced by BCG for NTM (20)

At present, the improvement of analytical techniques and the possibility of performing advanced tests in vivo have allowed us to go further in the investigation of cross-immunity.

A very recent study (February 2019), used various techniques to analyse this phenomenon. Initially, peripheral blood mononuclear cells (PBMC) of PPD positive individuals for *M. Tuberculosis* were selected and stimulated with BCG, MAV or MAB. After 7 days, they proliferated and expressed IFNγ or GZM-A, which were determined by flow cytometry. With this technique they found that the resulting T cells induced a cross reaction between *M. Tuberculosis* and MAC, in addition to both immune responses were comparable and similar.

Then, these BCG-specific T cells were cultured with autologous macrophages infected with MAV and MAB for an additional 3 days with the intention of measuring the cytokines resulting from differentiation into Th1, Th2 and Th17. In this second phase, the secretion of IL-17, IFN γ, TNFα and IL-6 that had initially occurred was highly increased, confirming once again this cross-immunity. The process is summarized in **Figure 9**

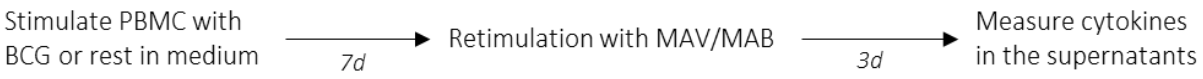

***Figure 9**. Schematic of experiments conduced to measure MAV and MAB cross-immunity*
PBMC: peripheral blood mononuclear cells; BCG: Calmette-Guérin Bacillus; MAV: M. Avium; MAB: M. Abscessus

To further elucidate the function of NTM-reactive T cells (CD4 + and CD + 8), their ability to inhibit the cellular replication of human macrophages infected by MAV and MAB was measured *in vitro*. It was found that BCG-stimulated T cells effectively inhibit the replication and growth of intracellular MAV and MAB much better than those with intracellular BCG. The inhibition is almost 80% for MAV and almost 60% for MAB.

Finally, to check the expansion of this cross-immunity, the vaccine was administered to mice in one or two doses, with a separation of 4 weeks between one dose and another. At the time of stimulating mice vaccinated with MAV or BCG antigens, the response was a significant increase in cross reactive splenic cells, compared to those subjects who were not sensitized.

This expanding effect of the response was **tested in human** subjects with no previous history of exposure to BCG antigens or a travel history to countries with endemic TB. Participants were administered one or two doses.

When stimulation with MAV or MAB antigens was performed, the response was the same as in murine models: **there was cross-immune response in both cases, with exponential growth of the reactive T cells in the case of those who had received a second dose**.

Implication of participating factors in the immune response

The grade of real implication of the different elements that compose the immunological reaction is unknown, although with available studies, a first approach can be made (20).

CD4+ T cells play an important role, since patients with low CD4+ counts (as in AIDS) develop more frequently disseminated forms of disease (40). Pulmonary forms by MAC are more frequent in patients without immunosuppression. In these clinical forms, high levels of CD4+, CD8+ and cytokines (TNFα, IL-1, IL-6 and IL-8) are found from alveolar secretions (41)

In fact, the number of CD4 + decreases as the bacterial load increases in these forms of the disease. It is believed that this happened due to an induction of apoptosis (42). Advanced forms of MAC have a decrease in Th1 response and low levels of IFNγ, which is usually partially reversible with antibiotic treatment (43,44).

However, the current management with antibiotic treatment is torpid and requires starting to look for alternative treatment techniques, guided directly at the host.

Through other studies, it has been proven that the **deficit of any of the participant factors of the immune response** (even if there is an isolated deficiency), **makes the infection much worse** (45–48). The deficit of T cells makes the action of effector cytokines unsustainable because MAV and MAB have the ability to decrease the response of macrophages and inhibit signal transduction (49,50).

4. JUSTIFICATION

Why is important this infection?

NTM lung infections are currently booming in our environment (2,3,51,4,6–12), especially in patients with a complicated baseline situation such as immunocompromised and with an underlying pulmonary disease (2,3,8,13,14,52,53).

Incidence in our environment, especially for MAC, has increased in the last 15 years (4) and, it could become a public health problem, mostly because their environmental origin and their ability to life in hot environments, as the drinking water network as the drinking water network (1,53).

These infections can cause a wide spectrum of affectations. While mortality rates are high in a short time period in case of pulmonary *M. Abscessus*, MAC does not lead to rapid dead (in 5 years, less than 20%). MAC produces a chronic lung disease with a deterioration of respiratory function, in patients that, already have an organic lung disorder. This worsens the quality of life of these patients, who die because of other illness comorbidities (54).

Why it is necessary to find another treatment?

Management of this infection is torpid and unsatisfying result due to several factors as 1) low cure rates, 2) toxicity of the antibiotic treatment, 3) easily appear resistance to treatment and 4) high proportion of reinfections.

Cure rates are low (normally around the 50%) because of the limited treatment options (5,20,22,55). The only treatment that has demonstrated a certain degree of efficacy is a triple antibiotic therapy, with Clarithromycin, Ethambutol and Rifampicin (9,15,23,25).

At the beginning, patients with pulmonary MAC where ineffectively treated with classical antituberculous drug, until DST where performed and they realized that this microorganism was resistant to most of antibiotics. Even so, DSTs are difficult to perform in these microorganisms, which adds difficulty to the selection of an appropriate treatment guideline (15,16,21,24,27,28).

High toxicity of the available treatment is another problem. Due to the long treatment duration, the probabilities to develop adverse effects are higher with drugs that already have an important toxicity. These adverse effects can be serious, especially in the liver and heart (5,15,23,29).

If patient heals, reinfection usually happens after the first 12 months since the negativization of sputum cultures. Almost always is due to another pathogen, even if it is the same species. Time between infections is relatively short, so if we add the pulmonary deterioration characteristic of the patient's underlying disease, respiratory recover is scarce. This occurs in the 20-40% of patients, according to some studies (20,27).

Why is BCG vaccine the treatment selected?

The use of BCG as an inductive treatment is proposed for several reasons. Epidemiology has shown that the incidence of TBC and NTM follow inverse trends in developed countries, and many studies have confirmed that there is a cross immunity that affects TBC and BCG versus MAC (20,28,33,56,57). Moreover, it has been demonstrated that BCG vaccine can stimulate an immune reaction that inhibits MAC replication within macrophages by almost 80% (20).

In addition, in some investigations they have seen that isolate alterations of cells or immunomodulatory components can make that the lung disease caused by MAC much worse (45–49), making the stimulation of a complete immunologic response more suitable in this case. This has also the advantage of archiving a certain degree of immunization against future infections.

Finally, the proposed treatment for this protocol is directed against a different target than the current antibiotic treatment. This would reduce the period of antibiotic administration and, therefore, the probability of developing adverse effects due to toxicity.

In conclusion, knowing that these pulmonary MAC infections incidence is increasing and that these infections worsen the life quality of the patients, it is necessary to find a more effective and less toxic treatment. The proposal would be to use the BCG vaccine in different doses, as a treatment-inducing immunotherapy to determine its efficacy based on the studies we currently have.

5. HYPOTHESYS

5.1. Main hypothesis

The induction of treatment with a BCG vaccine for pulmonary infection by MAC presents a dose response relation between 0.1mL and 0.2mL, so at a higher dose the proportion of patients that negativize the sputum culture is also higher.

5.2. Secondary hypothesis

Starting the treatment for MAC pulmonary disease in immunocompetent patients with BCG vaccine compared to placebo before the establishment of current multi-drug treatment is more effective than using only the standard treatment with triple-antimicrobials. It is related to:

- Faster microbiological cures
- Less reinfection at long term
- Fewer cases of adverse effects and toxicity of antimicrobial therapy.

6. OBJECTIVES

6.1. Main objectives

This study aims to determinate and compare effectiveness of induction of treatment with BCG vaccine to introduce it in the treatment of MAC lung disease in immunocompetent adult patients.

6.2. Secondary objectives

To achieve an increased cure rates, in immunocompetent adults with pulmonary disease due to MAC receiving BCG vaccine in comparison with patients that receive placebo:

- Microbiological samples cultured will be negative in less than 3-6 months since the beginning of antibiotic therapy.
- Less proportion of reinfections of the disease at 12 first months after negative culture, currently around 40%.
- Less proportion of toxicity due to the multi-drug treatment, currently around 36% (58).

7. MATERIAL AND METHODS

7.1. STUDY DESIGN

This is a randomized placebo-control parallel group clinical trial.

7.2. STUDY SETTING

The study has been designed as a multicentre study. It will be set among the following hospitals of Catalonia:

1. Hospital Universitari Doctor Josep Trueta, Girona (156.000 inhabitants as reference).
2. Hospital Universitari Germans Trias I Pujol, Badalona (800.000 inhabitants as reference).
3. Hospital Joan XXIII, Tarragona (600.000 inhabitants as reference).
4. Hospital Clínic, Barcelona (540.000 inhabitants as reference)
5. Hospital Universitari Arnau de Vilanova, Lleida (450.000 inhabitants as reference).
6. Hospital Universitari Vall d'Hebron, Barcelona (400.000 inhabitants as reference).
7. Hospital de la Santa Creu i Sant Pau, Barcelona (400.000 inhabitants as reference).
8. Hospital Universitari Bellvitge, Hospitalet de Llobregat (200.00 inhabitants as reference).
9. Hospital del Mar, Barcelona (350.000 inhabitants as reference).
10. Hospital de Santa Caterina (150.000 inhabitants as reference).

7.3. STUDY POPULATION

The study population will be composed by patients with pulmonary disease due to MAC (based on the diagnostic criteria established by the ATS 2007), that fulfilled the inclusion criteria and with any exclusion criteria on admission.

7.3.1. Inclusion criteria

1. Age ≥18
2. Only pulmonary disease
3. Immunocompetent patient
4. *M. Avium complex* as isolated germen: *M. Avium* or *M. Intracellulare*.
5. Not previously exposed to tuberculosis antigens:
    a. Not have suffered TBC
    b. Not have travelled or be natural from countries with endemic tuberculosis
    c. Not being vaccinated against TBC.

7.3.2. Exclusion criteria

1. Macrolide resistance
2. Sociopathy or other disabling not controlled psychiatric disorder.
3. Prior or current hepatopathy
4. Difficulties to attend the consecutive visits.
5. Immunosuppression:

   HIV, autoimmune disease or primary deficiencies, chronic diseases that require immunomodulator treatment (*peg corticosteroid*), malignancy and its treatment, and patient with previous transplantation.
6. Pregnancy

7.3.3. Withdrawal criteria

Patients presenting the following criteria would be withdraw of the trial:

1. Anaphylaxis due to the administration of the vaccine (20 minutes post-vaccination)
2. Any severe or life-threatening adverse event that could be related to administrated drug. This includes adverse effects of BCG vaccine and also if they are due to antibiotic guidelines.
3. Poor compliance of therapy after having been correctly trained by hospital staff involved in this clinical trial.
4. Dissemination of infection or appearance of other clinical form explained: adenitis or musculoskeletal

Withdrawn patients will not be replaced in the study and they will be included in the statistical analysis by (tècnica de l'arrossegament)

7.4. SAMPLE

7.4.1. Sample selection

Sampling process of this clinical trial will be a multi-staged sampling (also known as cluster) consisting in two stages.

Fist stage: convenience sampling.

This stage is based on choosing hospitals that will participate in our study by convenience. This first stage is done this way for practical reasons. The best way would be a randomized selection of hospitals, but it would be methodologically difficult to carry out.

So, assuming that all Catalan hospitals have the same means of care (in reference above all to diagnosis and treatment) and the population receives very similar assistance in all of them, we do not believe that selecting some of them is a source of bias of selection.

Second stage: non-probabilistic consecutive sampling.

The choice of patients accessing to the clinical trial is carried out based on the confirmation diagnosis of pulmonary MAC infection. Patients who arrive to the examination room (emergency service of other way) of selected hospitals are visited and submitted to the inclusion and exclusion criteria.

Those who meet these criteria and, after being appropriately informed, accept the informed consent, will be formally included in the study.

7.4.2. Sample size

Sample size will be calculated with GRANMO app. It is a free online software developed by Institut Hospital del Mar d'Investigacions Mèdiques.

Accepting an α-risk of 0.025 and a β-risk of 0.2 in a two-sided test, 108 exposed subjects in each group of vaccine and 108 in the non-exposed are necessary to recognize as statistically significant a relative risk greater or equal to 1.45. A proportion in the non-exposed group is estimated to be 0.5. It has been anticipated a drop-out rate of 10%.

We take into account a smaller alpha risk because intervention group will be divided in two different groups that will receive different doses, and it is necessary to decrease it.

If we rely on the review of recent studies, the cure of patients exposed to the intervention could amount up to 80% (taking into account the double therapy they will receive). For individuals not exposed to the intervention studied, the cure does not exceed 50% in most studies.

The low drop-out rate is estimated at 10%, because this aspect is controlled by two techniques: the first one is in the inclusion criteria, where patients that investigators have reasons to think that they will not have a good treatment adherence will be automatically excluded. The second is achieving this adherence by educating the patient in successive visits, where they will be trained and controlled. This estimated 10% will surely be due to cases beyond our control.

7.4.3. Time of recruitment

According to a descriptive study above-mentioned, Hospital Universitari Josep Trueta (Girona) attends around 12 patients suffering MAC pulmonary infection per year. So, taking into account the potential reference population attended in our 10 hospitals, we estimate that they attend approximately 299 patients with this disease annually.

As this trial consists of three different intervention groups, the sample will be 324 patients. Thus, estimating that approximately 10% of the candidates who meet the criteria will refuse to participate in the study and the drop-out rate will be also of 10%, the recruitment time will be 15 months.

7.5. STUDY INTERVENTIONS

7.5.1. Randomization and masking technique

The patients enrolled in this clinical trial will be randomized for their distribution in two main groups. Those who will receive the real intervention will be divided in two different groups for the administration of two different doses of vaccine:

- **Group A**: control group that will receive the intradermal placebo (**therapy A**).
- **Group B:** experimental group that will receive the BCG vaccine (**therapy B**).
    - **B-I:** this group will receive low dose of vaccine
    - **B-II:** this group will receive high dose of vaccine

Only two doses are going to be compared, since previous studies have administrated a maximum of 2 doses in human patients. Therefore, as the risk of presenting complications is dose-dependent, it has not been proposed to administer more dose of those studied before.

Population will be split in two groups to make a stratified random sample, according to the radiological pattern obtained in HRCT done for diagnosis:

- Patients with **fibrocavitary** disease.
- Patients with **nodular/bronchiectatic** disease.

This stratification will avoid the differences in efficacy of response to treatment due to the type of affectation.

Even though they are different, both clinical forms are treated in the same way, with the same antibiotics but at adjusted dose. Literature neither indicate differences in the cure rate between them, so is not expected to be a confounder factor. Likewise, this stratification will avoid a possible confusing effect and all groups are expected to be comparable.

Investigators will not intervene in assignation process, because members of each treatment group of treatment will be chosen randomly by a computer-generated randomization. Every new patient enrolled in study will be introduced in computerized system and it will show to the investigator the treatment group where the patient belongs to (**group A, B-I** or **B-II**), depending also on his/her radiological pattern. Using this technique, it is guaranteed that in all groups the representation is homogeneous.

This study will be double-blinded, so neither patient nor investigator will know which treatment is being administrated. It would not be necessary to use this masking technique

because the response of treatment is not based on subjective parameters, but in microbiological parameters. Using this technique, loss of follow-up will be avoided in patients who receive placebo, and on the other hand, we will also avoid possible performance bias by the investigator.

This masking technique can be performed even if we take into account that BCG vaccination usually causes a scar at the site of injection. This scar is not a marker of immunization and approximately 10% of vaccine recipients will not develop a scar. So, neither the patient nor the investigator will know what treatment has been administered (*Annex 4. Characteristics and safety of BCG*).

7.5.2. Study interventions

After the diagnostic confirmation and the informed consent, patients will be enrolled in the study. Patient will be divided in two groups, receiving both of them the same treatment guidelines.

Before starting the antibiotic treatment, two doses of the drug assigned to each group will be administered. They will be separated by a period of 4 weeks because vaccination guides establish this period between two live attenuated vaccines administration.

The vaccine doses administration will be separated in order to avoid the potential development of severe adverse effects and overdose in the participants. Side effects are dose-dependent, making not safety the entire vaccine administrations in a single dose.

The drugs administered in each group will be the following:

- **Control group** (Group A): they will receive placebo as treatment (**therapy A**). Intradermal injection **0.1mL of NaCl 0.9%.**
- **Intervention group** (Group B): they will receive the real immunotherapy BCG vaccine (**therapy B**). Intradermal injections of 0**.1mL of live attenuated BCG vaccine (Danish strain 1331, 2-8 x10_6 UFC in 1mL)**
    - **B-I:** one dose of each. First dose will be BCG vaccine and second will be placebo.
    - **B-II:** both doses will be real BCG vaccine.

The doses will be administrated by properly trained nurses of the participating hospitals. The vaccine will be administrated on the upper outer face of the arm (in the upper region of the distal insertion of the deltoid muscle). First dose will be administered in the left arm by convention and the second in the right, since it is not recommended to use the same arm in subsequent

vaccinations for at least 3 months from the administration of BCG (*Annex X. Characteristics and safety of BCG*).

During the next 20 minutes after administration, the patient will remain in observation at the examination room to make sure that he/she does not present allergic reactions to the administered compound. In the event that this occurs, the patient will be excluded from the study (*7.7.3. Withdrawal criteria*).

After administration of the two corresponding intradermal doses, the patient will start the standard treatment at home, with the oral antibiotic regimen recognized by the current guidelines. Despite not being the most effective treatment, it is the only with some proven efficacy.

- Nodular or bronchiectasis: Clarithromycin 1g + Ethambutol 25mg/kg + Rifampicin 600mg three times per week. To make it easy for patients, pills will be taken on Monday, Wednesday and Friday.
- Fibrocavitary: Clarithromycin 500mg-1g + Ethambutol 15mg/kg + Rifampicin 10mg/kg every day.

Patient weight to calculate the necessary doses of Ethambutol is needed. For clarithromycin dose we also need to know patient's regular medication and weight, although it does not adjust exactly. Weight will be obtained by a nurse measuring the patient weight in the examination room, after the administration of vaccine.

The chronic medication of the patient should be adjusted by the specialist who prescribes it for the duration of the antibiotic treatment, since it is a long period of time and the three prescribed antibiotics can reduce exposure to the patient's chronic drugs.

7.6. VARIABLES AND METHODS OF MEASUREMENT

7.6.1. Study outcome

The main variable is the therapeutic intervention: **dichotomous qualitative variable**

Immunotherapy will be introduced on the day of confirmation diagnosis, before the beginning of the antibiotic schedule:

- The control group (**group A**) will receive intradermal placebo (identified as **therapy A**).
- Both intervention groups (**group B**) will receive different doses of the intradermal BCG vaccine (identified as **therapy B**).

7.6.2. Dependent variables

Disease resolution will be the main dependent variable of the study: **dichotomous qualitative variable** (*Yes or No*). The measure to be used is the proportion of responses to each of the therapies received (**therapy A** or **therapy B**). What will determine this response will be the negativization of sputum cultures taken monthly to each patient from the day of diagnosis.

Secondary dependent variable:

- Time it takes to have a negative culture: It is a **discrete quantitative variable** and it will be measured in both groups (**group A** and **B**) by results of the sputum culture, counting all consecutive months in which the cultures have been positive for the presence of MAC.

- Re-infections in the first year after negativization of culture: It is a **dichotomous qualitative variable** (*Yes or No*). It is defined as the return of positive sputum cultures with or without the re-appearance of the initial symptoms (cough, dyspnoea, fever and / or weight loss) after a first negativization it in the following 12 months.
  Screening will be performed in the follow-up visit. Clinical recurrence will be detected by complete physical examination and collecting information about the possible symptoms manifested by patient. Microbiological recurrence will be detected by the positive result of sputum culture done in every visit.

- Complications related to antibiotic treatment: It is a **dichotomous qualitative variable** (*Yes or No*). It will be measured in both groups (**A** and **B**) as the apparition of adverse events linked to the toxicity of the substances. These are: gastrointestinal disturbances

(diarrhoea, nausea/vomiting, and abdominal pain), QT lengthening, hepatotoxicity, flu like syndrome and optic neuritis.

Screening of these symptoms will be measured in the follow-up visit by doing a complete physical examination and information about symptoms manifested by the patient, but also with analysis of hepatic function and with an EKG.

- Complications related to immunotherapy: It is also a **dichotomous qualitative variable** (*Yes or No*). Symptoms related with vaccine are the following: suppurative lymphadenitis, headache, fever, ulcer at the injection site, acute or chronic inflammation of the bones (due to infection or not), inflammation with pus from the nodes, abscess in the injection area. Although adverse effects are not very common, screening of these symptoms will be measured in the follow-up visit by doing a complete physical examination and information about symptoms manifested by the patient

7.6.3. Covariates

The following variables will be taken into account in order to avoid possible confounding action:

- Age: it is a **continuous quantitative variable**. It will be obtained from patient's ID card or other in force document by calculating through the year of birth. Expressed in years.

- Gender: it is a **dichotomous qualitative variable** (*male or female*). Also obtained from patient's ID card or other in force document.

- Presence of underlying pulmonary disease: It is a **dichotomous qualitative variable** (*Yes or N*o). It is described as the presence of one or more of the following diseases: COPD, bronchiectasis, emphysema, asthma and obstructive sleep apnoea.

- Time from the beginning of symptoms until the start of treatment: It is a **continuous quantitative variable**. It will be collected by consulting the clinical history of patient and asking patient. Expressed in *days*.

All variables are summarized in **Table 5**

Table 5. Variables of the study

	Variable	Type	Categories	Measure inst.
Independent	*Therapeutic intervention*	Dichotomous qualitative	Group A/B	
Dependent	*Disease resolution*	Dichotomous qualitative	Yes / No	Sputum culture
	Time negativization culture	Discrete quantitative	Months	
	Recurrence of infection	Dichotomous qualitative	Yes / No	Sputum culture + clinical evaluation
	Complications of antibiotics	Dichotomous qualitative	Yes / No	Clinal evaluation + hepatic function + EKG
	Complications of immunotherapy	Dichotomous qualitative	Yes / No	Clinical evaluation
Covariates	*Age*	Continuous quantitative	Years	Clinical examination + patient's history
	Gender	Dichotomous qualitative	Male / Female	
	Underlying lung disease	Dichotomous qualitative	Yes / No	
	Time from the beginning of symptoms until treatment	Continuous quantitative	Days	

7.7. METHOD OF DATA COLLECTION

All the information that should be recorded appear in the *Annex 1. data collection sheet*.

Every patient under suspicion of pulmonary mycobacteriosis that attended the selected hospitals must be admitted, and remain in respiratory isolation, until the presence of TBC is ruled out. The objective is to make a differential diagnosis between active TBC, lung malignancy and nontuberculous mycobacteria. Since pulmonary MAC infection is an exclusion diagnosis, it is necessary to perform some previous steps (3. Introduction: diagnosis). These tests only ensure the inclusion criteria to our study.

In the exclusion criteria, there is “resistance to macrolides”. This is important because DST is not routinely practiced at the beginning of treatment due to its low profitability. To find our patients, and to make sure they don't have any exclusion criteria, an antibiogram and a liver function test will be performed. The rest of criteria will be checked through the anamnesis and patient’s medical history.

Once TBC is ruled out, it is not necessary for the patient to remain in the hospital. The rest of the process will be ambulatory.

If the patient accomplishes all the study criteria, he/she will be duly informed and will sign the informed consent to be included in the study. In the same visit, treatment will begin according to the therapy group where the patient has been randomly assigned (group A placebo and group B BCG vaccine).

Both therapies will be administered in the examination room by the trained nurse, and patient will stay in this area for at least 20 minutes monitoring the tolerance to vaccine in case there was an anaphylactic reaction. In this case, it would be a withdrawal criterion.

After that time, patients can return home until the next month (4 weeks after the first dose) to be given the second dose of treatment assigned. The process will be the same as in first dose.

The day of the administration of the second dose, an antibiotic guideline will be established for all patients depending on their radiologic pattern (explored in diagnosis by HRCT). Patients will perform this stage of treatment at home.

While antibiotic treatment persists, hepatic function and EKG will be performed in both groups every two months to assess pharmacological toxicity. This will be performed by laboratory staff and an experienced nurse, who will notice the patient to program a visit in case of alterations of any parameters.

In this way, an exhaustive clinical evaluation will be performed in addition. It will evaluate how patients are feeling with the treatment and it can be giving advices about the identification of possible toxic effects or lack of response to the antibiotic treatment. In this case, after 6 months of multi-drug guideline it should be reassessed. If any disease reappearance or persistence doubt exists, complementary techniques of diagnosis will be carried out. Developing toxicity because of antibiotics will be a withdrawal criterial too.

In order to evaluate the study outcome (disease resolution), a sputum culture will be obtained in all patients of both groups every month since the antibiotic treatment is established.

The same culture results will be suitable to measure other two dependent variables as time to negativization of the cultures and recurrence of the disease.

Drug compliance checking in both groups will be carried out in the follow-up visits by requesting the patient to bring the pills. In the visit we can ensure that the pills are being taken correctly. In case of patient does not express clearly his treatment's adherence, a urine sample may be requested at the same visit. This will be useful to check the orange staining due to Rifampicin intake. As it mentioned above, the responsible infectologist will ask the patient about compliance problems and possible adverse drug events, which are the most important adherence factors.

Finally, in this way, the informed consent will contain a section in which patient agrees to accomplish the treatment as it is established in this protocol.

The follow-up will last approximately a half and a year (18 months) for all groups because with current treatment cultures tend to negativize in 3-6 months. After that, oral treatment should last at least 12 more months. This follow-up visits will take place every 4 weeks, where the previous evaluations will be carried out (complete clinical examination, evaluation of symptoms and reading of the result of the previous month's culture).

These actuations will be carried out by an internist infectious disease specialist. All interventions to perform are listed in **Table 6**, assuming the maximum time of follow-up.

Table 6. Plan of interventions. *IC: informed consent, HRCT: high resolution CT, EKG: electrocardiogram*

Diagnosis		Randomization	Follow-up visits (months)																	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Inclusion criteria																					
Inform and IC																					
Recollection of sputum/culture																					
HRCT																					
Hepatic analysis + EKG																					
Intervention																					

8. STATISTICAL ANALYSIS

Statistical analysis will be completed using the Statistical Package for the Social Sciences (*SPSS Windows®*) by the responsible statistician. It will be performed in two different ways:

- Intention to treat analysis in order to not underestimate the adverse effects of the treatment. It confers a more realistic vision since it maintains the balance achieved with the randomization to make the four groups comparable to each other. It will include all initial patients enrolled on the study, even if they have not fulfilled the requirements of the protocol and have not completed the study.
- Per protocol analysis: not necessary because it is not a non-inferiority clinical trial, but increases external validity. In this analysis the only included patient will be the ones who have fulfilled the requirements of the protocol and completed the study.

If these two ways of analysis yield the same conclusions, the confidence in the results is magnified exponentially. For drop-outs, last observation carried forward will be used in successive controls until the end of the study.

8.1. Statistical plan

The following section describes the items that will be necessary to evaluate and relate in order to analyse the data collected from each patient throughout the study:

1. Description of sample will be performed with the sociodemographic variables (age and gender), but also in relation with study outcome (negativization of culture) and the other dependent variables (time to negativization of culture, re-infections in the first year, complications related to antibiotic treatment and complications related to immunotherapy).
2. The study outcome (negativization of culture) will be compared with the intervention (BCG vaccination vs placebo) aiming to establish a relation between them.
3. We will also need to quantify the difference of study outcome (difference of response to treatment) in all groups (group A, group B-I and group B-II).
4. Secondary dependent variables will be compared with the intervention in order to establish a relation between them.
5. Although it is a clinical trial, where the sample has been stratified and randomized, an analysis of the covariates will be performed to avoid any confusing effect that may occur on the results.

8.2. Univariant analysis

The result of the variables in each group of study will be expressed according to if they are qualitative or quantitative in order to describe the sample:

Qualitative variables: These variables will be expressed as a proportion (%), that will be represented as a table of frequencies and a sector diagram.

- Therapeutic intervention
- Disease resolution
- Recurrence of the infection
- Complications related with antibiotic administration
- Complications related with immunotherapy
- Presence of underlying pulmonary disease
- Gender

Quantitative variables: results for variables with a normal distribution will be expressed as a mean with a standard deviation (SD). Those without a normal distribution will be expressed as a median with estimated quartiles.

- Age
- Time to negativization of the culture
- Time since the apparition of symptoms until treatment.

To check the distribution of the sample, the choice test will be Kolmogorov-Smirnov.

8.3. Bivariant analysis

To analyse the association between the independent variable and the dependent variables different tests will be used.

- Chi-square to evaluate two qualitative variables, which are therapeutic intervention and disease resolution (dichotomous qualitative variables). Also, recurrence of infection and complications related to antibiotics will be evaluated with this test. This will indicate the probability that the results obtained are due to chance.
- Relative risk (RR) will reflect the excess risk in the group exposed to the vaccine (group B) compared to the group not exposed / exposed to placebo (group A).
- ANOVA will be useful to relate a qualitative variable (therapeutic intervention) with a quantitative variable (time to negativization of culture). That is in the case of 3 different groups and in the assumption that the distribution of the sample was normal. If there

were only 2 groups, T-student will be the election for this analysis. In case of not normal distribution, the election would be Kruskal-Wallis.

Results will be considered statistically significant if p-value is <0.05 in a defined confidence interval of 95%.

8.4. Multivariant analysis.

Multivariant analysis is not much necessary in clinical trials because these studies are controlled to avoid possible confounding effects. In this case, main confounding factor could be radiological patterns, but it has been controlled by stratifying our sample.

If it was necessary, we would apply Multiple Logistic Regression model for qualitative variables, and Lineal Regression model for quantitative variables.

9. EHTICAL CONSIDERATIONS

This clinical trial will respect the medical ethics principles of human experimentation, according to the **World Medical Association Declaration of Helsinki (1964)** (59) about the Ethical Principles for Medical Research Involving Humans Subjects (last actualization October 2013).

Once this protocol will be finished, it will be sent to the **Clinical Research Ethics Committee (CEIC) of the Hospital Universitari de Girona Doctor Josep Trueta**, Girona. The validation by this committee is mandatory to start the clinical research, as it is registered in the "**Real Decreto 1090/2015, de 4 de diciembre, ensayos clínicos con medicamentos** "(60). In this way, permission will also be solicited to the direction of each of our hospitals and the protocol will be sent to the "**Asociación Española de Medicamentos y Productos Sanitarios (AEMPS)"** to receive its authorization. After the approval, the last step will be submitting the protocol to the European Clinical Trials Database (EudraCT).

About this legislation, it is also important to consider the obligation of an economical compensation to the patients if they suffer injuries due to the clinical trial, being needed an insurance to face with these situations.

In addition to the CEIC, the management of the centres that enter in the study will have to approve it as well. To participate in the study, it is mandatory that patients read and understand the **information sheet** (*Annex 2* (61)) of this clinical trial, so they can be able to sign the **informed consent form** (*Annex 3*). This way, the principle of autonomy will be respected. The protection of the rights, safety and well-being of the patients participating in the study is guaranteed.

To ensure the confidentiality of the information regarding the identity of the subjects involved in this trial, the "**Ley Orgánica de Protección de Datos Personales y Garantía de los Derechos Digitales 3/2018"** (62) must be accomplished.

Taking into account the ethical principles, we decided using placebo to make the comparison in our study because all patients will be receiving concomitantly the usual authorized antibiotic regimen, so that none of them will be left untreated.

Ethically, there are no contradictions in delaying the start of antibiotic treatment, since MAC lung disease is a slow and progressive infection, with only notable mortality in cases of immunosuppressed patients. It cannot be treated based on a suspected diagnosis, but it is necessary to wait for the definitive diagnosis and the results of the antibiogram. Clinical practice guidelines determine this because of the ease of these microorganisms in developing drug resistance. All the investigators will have to declare no conflict of interest.

10. STUDY LIMITATIONS

This type of Mycobacteriosis (MAC lung disease) is a low incidence disease, so the recruitment time would be long. To minimize this circumstance, a multi-centre test is planned. This type of study can generate procedural variability between some hospitals and others, so that all researchers who are going to influence the clinical trial will be previously instructed. Likewise, initially it has been assumed that in the case of hospitals in Catalonia, the care received, and the procedures performed will differ little from one another.

Even assuming that the variability between the different hospitals will be small and given that many healthcare centres will participate, it will be necessary to emphasize another procedure intended to minimize the variability of results. This will be the training of participants. In this way, instructional sessions will be held in all selected hospitals.

They will explain in detail the operation of the clinical trial and all the steps to follow during it, in addition to providing specialized training to each participating department: nursing will be instructed on the administration of the vaccine and the examinations to be performed during the tracing. These sessions will aim to unify procedures to follow.

In addition, this disease is easily confused with others, the inclusion/exclusion criteria for the study are strict and the diagnostic criteria for the disease are those standardized by ATS / IDSA.

Although our procedure could be considered expensive, we consider that a randomized clinical trial is the best design to achieve the objectives of our study. In addition, it is important to know that, if our hypothesis is confirmed, BCG-induced treatment would reduce the time of antibiotic treatment. This would reduce the spending on drugs, and their monitoring and assistance in case of intoxication. This control, despite being infrequent, could require expensive and invasive procedures with hospitalization.

A prospective study like this has the added risk that some patients will be lost due to lack of compliance, drug toxicity or loss of follow-up due to the length of the same. To address this problem, an abandonment rate has been estimated in the calculation of the measurement of the sample. In addition, certain factors (i.e. disabling mental illness or lack of commitment) to screen potential patients with follow-up difficulties have been included in the selection process. This loss of follow-up is also avoided thanks to the application of masking techniques, explained below.

Since the evaluation of our intervention will be carried out by microbiological and non-subjective parameters, it would not be necessary to apply a masking technique, but we decided to carry out a double-blind clinical trial because by doing so, in addition to avoiding patient

abandonment who know that they have received placebo, we will avoid the performance bias by the researcher.

Our main dependent variable (disease resolution) includes the negativization of sputum cultures as a measurement parameter. Thus, although the patient may require concomitant supportive treatments for the symptoms of the disease, such as dyspnoea, fatigue, cough or fever, its administration would not influence the results of this culture.

Both therapy groups in our study will have to take medications at home, so therapeutic compliance must be strictly controlled. To deal with this circumstance, some evaluations will be established:

- Informed consent (see *Annex 3*): it will contain a section in which the patient agrees to perform the treatment as established in our protocol.
- During follow-up visits: the patient will be asked at each visit to bring the medication to make sure it is taken correctly, counting the pills that have been consumed. Just in case of patient does not express clearly his treatment's adherence, a urine sample may be requested at the same visit. In addition, during these follow-up visits, the responsible internist will talk with the patient about compliance problems and possible adverse effects of the medications, which is the most important factor for adherence.

Randomizing the sample is a way to control the possible confusion. In addition, we have included a stratification by radiological pattern of the different types of disease. So that, patients are distributed homogeneously and randomly among the intervention groups.

Another variable that could create confusion in the results is the presence of underlying lung disease (COPD or bronchiectasis), which has been controlled by including it in the covariates specified in section "*7.5.3. Covariates*".

A poorly developed technique can lead to alterations in its therapeutic effectiveness in these patients. With this in mind, we will have to make sure that the vaccination technique is the correct, and the same, for all the nursing team that will carry them out. This is complicated to measure and, therefore, we must bear in mind that differences between them will be inevitably.

To limit this bias, in the months prior to the start of patient recruitment, training will be conducted for participating staff, to ensure that the technique is correct. It will be carried out by a nurse who has extensive experience in administering this vaccine.

It is important to highlight that being a multicentre study implies more coordination and greater efforts to ensure quality in relation to recruitment, treatment and monitoring. But, on the other hand, the representation of the sample and the equipment involved are higher than in a single-centre study, which gives more external validity.

11. WORK PLAN

Tasks of coordination, interpretation and presentation of results will be developed by the research team. **Infectiology specialist of internal medicine** will be the main investigators team in each hospital. In these groups there will be a coordinator, who will meet twice a year with the other coordinators.

To perform the study, it will be necessary to consider other investigators in each hospital as a **pharmacist** to provide the drugs, a **radiologist** to do HRCT, a **laboratory staff** to analyse cultures and analytics, a **nursing staff** and one **statistic** to analyse the results.

11.1. Sequence

Stage 0 (M1-M3[1])

September - December 2019. Study design and ethical evaluation. Investigators will be the main responsible.

1. **Bibliographic research and protocol elaboration:** This are the first steps to carry out this clinical trial. Development begins with formulation of a study aiming to answer the hypothesis formulated after an extensive bibliographic research. After that, variables will be defined, and methodology will be established

2. **Presentation and evaluation** of the protocol by the **Clinical Research Ethics Committee** of Hospital Universitari Doctor Josep Trueta (Girona).

3. Contracting an **insurance**.

Stage 1 (M4-M5)

January – February 2020. Meeting of research team and training. Investigators and co-investigators will be the main responsible.

1. **First meeting of research team**: choose who will be the main investigator of each hospital included in the clinical trial. Coordinators will meet twice a year to evaluate if protocol is being well fulfilled. If something is not working, they will take the necessary decisions.

[1] *M: Month. Makes reference to each period of time included in different stages of the study*

2. **Multidisciplinary team meetings**. Organization of tasks, how to fill the data information sheet and sequence of data transference will be included.

3. **Training**: Internists who will participate in the study will be teach about the study protocol in order to avoid differences when assistance (diagnosis and treatment). They will be instructed in collecting and registering data, giving information to patients and diagnosis and treatment of MAC. Also, nurses will receive training about correctly administration of BCG vaccine.

Stage 2 (M6-M39)

March 2020 – November 2022. Sample collection, data collection and follow-up visits. Investigators and co-investigators will be the main responsible.

1. **Patient recruitment**: patients will be enrolled in the study by consecutive sampling once we ensure they accomplish the inclusion and exclusion criteria and if they accept the informed consent. This process will last for 15 months.

2. **Follow-up visits**: during the treatment, patients will be controlled every 4 weeks since the onset of the treatment to evaluate the response and possible adverse events.
   Follow-up will last 12 months after negativization of culture of sputum (it happens mostly 3-6 months of treatment), so we start take the maximum follow-up time necessary that will be 18 months.

3. **Data collection**: each internist will record the information collected in every visit in the database by using data collection sheet (*Annex 1: data collection sheet*).

4. **Coordinators of each hospital will meet twice a year** to evaluate if the protocol is being well fulfilled and if it is not, this council will take the necessary decisions.

Stage 3 (M40-M41)

December 2022 to January 2023. Data analysis and interpretation. Coordinators and the statistic are main responsible.

1. **Statistical analysis**: All collected information will be analysed by an experienced statistical according to the variables of our trial.

2. **Results**: interpretation of results and elaboration of discuss and conclusions.

Stage 4 (M42)

February 2023. Publication of result. Coordinators are main responsible.

1. Principal investigators will **generate a paper** to show the study result and conclusion.

2. This article will be sent to **"Enfermedades Infecciosas y Microbiología Clínica",** which is the official publication of "Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC)".

Table 7. Workplan

Task	2019				2020												2021												2022												2023	
	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
Stage 0: Study design and ethical evaluation																																										
Bibliographic research																																									
Protocol elaboration																																									
CEIC/AEMPS																																									
Insurance contract																																									
Stage 1: Meeting of researchers and training																																									
Meeting of researcher																																									
Multidisciplinary team meetings																																									
Training																																									
Stage 2: Sample collection, follow-up visits and data collection																																									
Recruitment and data collection																																									
Intervention																																									
Follow-up																																									
Stage 3: Data analysis and interpretation																																									
Statistical analysis																																									
Interpretation results																																									
Stage 3: Publication of results																																									
Writing of paper																																									
Publication																																									

12. BUDGET

Non-included costs

Staff: Health personnel participating in the study have been counted with no additional cost because they work at the hospital.

Materials: Diagnostic techniques are not included in this budget, since they are part of the usual procedure in case of pulmonary MAC (ATS / IDSA recommendations). Neither antibiotic treatment is included, since it is the only treatment of which It is currently available and therefore, is the standard guideline that we would use outside the clinical trial to treat these patients.

Personnel recruitment

A qualified statistician will be hired for data managing and statistical analysis, and a project manager for monitoring and revising data collection will be hired to ensure data validity.

Insurance policy

As this study is an open-labelled clinical trial, an insurance will be contracted for safety.

Material needed:

We have taken into account that the material expenses will include those materials used in the intervention, those that are not usually part of the treatment protocol for these infections.

So, we will take into account the doses of vaccine and placebo that we will administer, as well as the DST that we will perform to our patients to ensure that they meet the inclusion criteria to the study. This technique is not included in the systematic management of MAC pulmonary infection unless there is a lack of response to the antibiotic treatment, so we have included it.

Results publications, divulgation and travel

Seven meetings will be held throughout the study to coordinate the 10 hospitals that participate in it. The hospital coordinator (principal investigator) will attend those meetings.

A cost of 100€ has been estimated with in diet and travelling concept.

Attendance at two conferences (national and international) to present the results have also been included in the budget.

Item	Hours or units	Unit cost	Subtotal
Staff expenses			**0€**
Infectologist		0€/h	
Nursing staff		0€/h	
Pharmacist		0€/h	
Radiologist		0€/h	
Laboratory staff		0€/h	
Subcontracted professional services			**19.200€**
Qualified statistic	8h/d - 3d/w - 4w	35€/h	3.360€
Project manager	4h/d - 1d/w - 33m	30€/h	15.840€
Insurance policy			**16.200€**
Trial policy	324	50€/patient	16.200€
Material			**6.039,36€**
BCG vaccine	324 doses	1,16€/dose	375,84€
Placebo NaCl 0.9%	324 doses	0,30€/dose	97,20€
DST: microdilution gram+ bacteria	324	16,28€/patient	5274,72€
Documentation printing - *Data collection sheet* - *Information sheet* - *Informed consent*	9 pages/patient	0,10€/copy	291,60€
Fees, publication and travels			**11.114,55€**
AEMPS fees	1	114,55€	114,55€
Revision and publication fees	1	1.500€	1.500€
Coordination meetings	7 meeting / 10 coord.	100€ meet/coord.	7.000€
National congress	1	500€	1.000€
International congress	1	1.000€	1.500€
		TOTAL	**52.553,91€**

13. FEASIBILITY

Developing this protocol, actions have been planned in order to ensure the feasibility of the project.

First, was important to optimize as closely as possible the time of study. Incidence of MAC infection, while booming, still being low and the required sample is 324 patients, so the recruitment time would be long if ten hospitals were not included in our study.

The hospitals have been selected based on the reference population they provide. Reference hospitals of four Catalan provinces have been chosen in order to ensure the smallest variability in a reference population and being as representative as possible.

In this way, we have achieved to reduce time of recruitment to one year and three months, taking into account the drop-out and the patients who refuse to participate. Even though we have not included many hospitals to avoid bias on our results.

In addition, all selected hospitals are from Catalonia, so it is assumed that the resources and assistance given to all patients will be the same. If it was not, sessions of training and sharing of the protocol of the study have been programmed.

This study is lengthy in time due to the long follow-up in each participant (up to 18 months). To make it shorter, it would be necessary to involve more hospitals, although this may increase the risk of bias.

Regarding resources, most of procedures are the usually carried out in the management of these patients, so it will not have a very high additional cost for the system. Techniques used are also routine and easy to carry out by the personnel of selected hospitals. Also, in this sense, it is a feasible study.

To make a summary, the study has been designed in a way that is feasible in time, costs and resources.

14. IMPACT ON THE NATIONAL HEALTH SYSTEM

NTM infections are currently increasing their incidence due to several factors, such as increased host susceptibility (associated with immunodeficiency and interstitial lung diseases) or the continuous improvement in diagnostic techniques.

Despite not always being associated with notorious mortality figures (in case of *M. Avium Complex*), NTMs constitutes a public health problem given its form of transmission. Because of this, some internal medicine specialists consider that, over time, they will become mandatories of systematic reporting.

These changes in its epidemiology have made them become a new concern and its torpid management has forced the research for new ways to treat, not merely limited to the usual antibiotic treatment. For example, novel treatment manners may include immunotherapy.

In this regard, the proposed clinical trial provides a new source of treatment that has nothing to do with traditional antituberculotic drugs and that could have a positive impact on the treatment of these patients by: increasing cure rates, decreasing the toxicity caused by antibiotics, and reducing the possibility of future reinfections.

These improvements will translate into a better prognosis in all aspects for the patient and a reduction in costs for the system, as they do not need to administer antituberculotic drugs for so long and do not need to carry out toxicity monitoring tests.

In addition, these complications involve hospitalization of patients, some with serious effects (e.g. hepatitis or electrical abnormalities of the heart) will also be reduce.

If the currently study demonstrates good effectiveness of the proposed treatment, it will be necessary to perform some other studies before the standardization of BCG as an immunologic treatment for pulmonary MAC.

Future studies will need to prove the effectiveness of proposed treatment in other points of view that have not been measured in this study, as improvement of symptoms or radiologic sequelae. This is due to the presence of underlying pulmonary disease in patients who develop this infection, because in this context is common to suffer also an exacerbation of their basis pathology. So, it would be difficult to measure symptoms and its improvement and discern between clinical manifestations of basis pathology versus infection.

The same occurs in the case of radiological sequelae, which, due to the patient's pathology, is difficult to discern if the increase of lung lesions is due to the affections of their basis disease or is due to the infection, as it is chronic course infection.

It will be interesting compare the response to this proposed treatment in the two main different clinical forms. Dose of antibiotic administrated is different in fibrocavitary and nodular-bronchiectatic disease and could also be different in this new immunologic treatment.

In conclusion, this protocol proposes a new way of treatment for infections that have a torpid evolution with the currently treatment. If the proposed treatment works as well as it is believed, patients with pulmonary MAC will have a real chance of healing. It would reduce even toxicities and reinfections they usually suffer and could improve their quality of life after the infection.

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ANNEXES

Annex 1. Data collection sheet

Hoja de recogida de datos:	Proyecto:
Infección pulmonar por *M. Avium Complex*	Introduction of BCG vaccine as immunotherapy on pulmonary disease due to *Mycobacterium Avium Complex:* effectiveness determination (II phase)

***Instrucciones**: Para la participación del paciente en este proyecto, es fundamental que cumpla <u>TODOS</u> los criterios de inclusión y <u>NINGÚN</u> criterio de exclusión*.

Criterios de inclusión		Criterios de exclusión	
Germen aislado MAC		MAC resistente a macrólidos	
Edad ≥18		Sociopatía	
Sólo forma pulmonar		Hepatopatía previa o actual	
Inmunocompetente		Dificultades para asistir a visitas consecutivas	
Sin exposiciones previas a antígenos TBC*		Embarazo	
		Inmunosupresión**	

** No haber sufrido TBC, no haber viajado los últimos 6 meses a países con TBC endémica ni ser natural de ellos, no haber sido vacunado contra TBC.*

*** VIH, enfermedades autoinmunes o deficiencias primarias, enfermedades crónicas que requieran tratamiento inmunomodulador, neoplasias activas o en tratamiento y trasplante de órganos sólidos o hematológico previo.*

Datos generales							
Apellidos				**Nombre**			
Fecha nacimiento	/ /	Día inclusión	/ /	Género	Hombre ☐	Mujer ☐	
Hospital							

Historia clínica		
Alergias		
Medicación habitual		
Antecedentes patológicos		
Antecedentes quirúrgicos		
Clínica respiratoria		
Constantes vitales	Frecuencia cardíaca: lpm	Temperatura: ºC
	Frecuencia respiratoria: rpm	Saturación O_2: %
	Tensión arterial: / mmHg	
Peso y talla		

Información relevante para el ensayo al inicio	
Tiempo desde el inicio de los síntomas	
Síntomas de enfermedad	
Informe TACAR	
Informe cultivo de esputo	
Función hepática	
Informe ECG	
Resistencias antibióticas	

Parámetros por considerar en la administración de la intervención (vacunación)		
Síntomas sugestivos de reacción adversa*	Primera dosis	
	Segunda dosis	

** A controlar durante los 15-20 minutos iniciales post-administración.*

Información relevante en las visitas de seguimiento		
Exploración física		
Adherencia al tratamiento		
Parámetros analíticos	Esputo	
	Función hepática	
	ECG	

Annex 2. Information sheet

HOJA DE INFORMACIÓN SOBRE EL ENSAYO CLÍNICO

Centro asistencial: _____

Investigador principal: _____

Nos dirigimos a usted para informarle sobre un ensayo clínico en el que se le invita a participar. El estudio ha sido aprobado por el Comité Ético de Investigación con medicamentos correspondiente y la Agencia Española del Medicamento y Productos Sanitarios, de acuerdo con la legislación vigente, el **Real Decreto 1090/2015, de 4 de diciembre**, por el que se regulan los ensayos clínicos con medicamentos.

Nuestra intención es tan sólo que usted reciba la información correcta y suficiente para que pueda evaluar y juzgar si quiere o no participar en este ensayo. Para ello lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir después de la explicación. Además, puede consultar con las personas que considere oportuno.

1. Participación

Debe saber que su participación en este estudio es voluntaria y que puede decidir no participar y retirar el consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni se produzca perjuicio alguno en su tratamiento.

2. Descripción del estudio

Las infecciones pulmonares por *M. Avium Complex* son entidades que en los últimos años han aumentado de forma considerable su incidencia y, pese a no ser letales en la mayoría de los casos, sí que conllevan grandes alteraciones orgánicas a nivel pulmonar que provoca un importante deterioro crónico de la función respiratoria. El principal factor que predispone, y a la vez contribuye a su gravedad es, en este caso, la enfermedad pulmonar subyacente que presentan muchos pacientes (EPOC, asma, fibrosis quística…).

Actualmente, el tratamiento antibiótico del que se dispone no es muy efectivo y el número de pacientes que se curan totalmente es reducido (55%). Además, se acompaña de un gran número de reinfecciones (hasta el 40%) y toxicidad farmacológica debido al largo período de tratamiento que se precisa (12-18 meses).

Es para evitar todos estos efectos secundarios, reinfecciones y mejorar la tasa de curaciones que se ha desarrollado este nuevo protocolo de tratamiento, basado en resultados de estudios recientes que son favorables a incluir esta vacuna en el tratamiento de las infecciones pulmonares por *M. Avium Complex*. Este protocolo consiste en la combinación de terapia inmunológica por medio de una vacuna (BCG) y antibióticos habituales.

Objetivo:

El principal objetivo del ensayo es determinar la dosis más adecuada de vacuna a administrar para tener un mayor efecto y una menor toxicidad. Así, al poder disminuir la pauta de antibióticos, se disminuirán los efectos tóxicos del mismo. Además, el efecto inmunizador de la vacuna disminuiría el número de reinfecciones posteriores al tratamiento de la primera infección. Finalmente, se espera que el número de pacientes curados ascienda por encima del actual. Se analizarán también las complicaciones derivadas del tratamiento con dicha vacuna.

Metodología:

Este ensayo incluirá pacientes adultos inmunocompetentes con infección pulmonar causada por *M. Avium Complex.* Antes de considerar su inclusión a este estudio, le ha sido realizada una batería inicial de pruebas consistente en un TAC torácico de alta resolución, una obtención de muestras respiratorias para ser cultivadas y analizadas sus resistencias a los antibióticos, una analítica de sangre que incluirá un perfil de función del hígado y un electrocardiograma. Todas estas pruebas están destinadas al diagnóstico definitivo de la infección y a detectar posibles futuras complicaciones o afecciones que impedirían la correcta medicación del paciente.

Los pacientes del estudio se distribuirán de forma aleatoria en dos grupos de tratamiento (A y B), estratificados en función del patrón de lesiones pulmonares radiológicas que presenta. Los dos grupos recibirán el tratamiento antibiótico habitual que se considera estandarizado para este caso. Además, el grupo A recibirá 2 dosis de un tratamiento endovenoso en forma de placebo. El grupo B estará dividido en 2 subgrupos que recibirán diferentes dosis de la vacuna BCG. Las dosis en los dos grupos serán administradas una vez al mes y será al finalizar estas vacunas cuando los pacientes inicien el tratamiento con antibiótico, ya que el tratamiento de estas infecciones pulmonares no es urgente. De no ser así, los antibióticos podrían interaccionar con la vacuna, disminuyendo su eficacia. Cabe destacar que, durante este proceso, ni el investigador ni usted conocerán el tipo de fármaco que se le ha administrado por vía endovenosa (no conocerán si recibe placebo o vacuna BCG).

Las visitas de seguimiento se llevarán a cabo una vez al mes, y en ellas se realizará una exploración física minuciosa para evaluar la evolución de los síntomas propios de la enfermedad y aquellos que podrían indicar una reacción adversa al tratamiento. También se extraerá una vez al mes una muestra de contenido respiratorio para su cultivo que permitirá evaluar la respuesta al tratamiento administrado. Cada dos meses realizaremos una analítica de sangre con perfil hepático y un electrocardiograma para monitorizar la toxicidad producida por los antibióticos.

Las evaluaciones previstas dentro del manejo estándar son las estipuladas en este protocolo. Este durará 12 meses más desde el momento en que los cultivos del contenido respiratorio sean negativos. Con el tratamiento habitual y teniendo en cuenta las variaciones propias de cada paciente, este proceso oscila entre 12 y 18 meses.

3. Beneficios y riesgos asociados a la participación del estudio

El fármaco estudiado en este ensayo clínico (vacuna BCG – Bacilo Calmette-Guérin) está comercializado actualmente y se utiliza en la inmunización contra la Tuberculosis y como tratamiento inmunológico adyuvante en el cáncer de vejiga, si bien es cierto que nunca se ha utilizado para inducir el tratamiento de la infección pulmonar por *M. Avium Complex*.

A pesar de ello y dado los problemas de manejo terapéutico derivados del tratamiento antibiótico, se han realizado recientemente estudios que demuestran un efecto de inmunidad cruzada entre la tuberculosis y estas micobacterias, sugiriendo que sería posible introducirla como tratamiento inicial antes de la administración del antibiótico y obtener unos resultados mejores a los obtenidos con el tratamiento oral únicamente (en varios aspectos como tasa de curación, reinfección futura y toxicidad).

En cualquier caso, las indicaciones iniciales de este fármaco no contemplan la posibilidad de inducir el tratamiento de estas infecciones, así que se han previsto unos criterios de inclusión y exclusión al estudio muy estrictos, de forma que sólo aquellos casos con enfermedad pulmonar sin otras complicaciones y con unas buenas perspectivas de tolerancia al tratamiento son los pacientes incluidos en el ensayo clínico.

Finalmente, para evitar riesgos asociados, se le informará sobre los posibles signos y síntomas que indicarían un empeoramiento de la enfermedad. De igual forma, se realizarán los estrechos controles estandarizados de toxicidad para el tratamiento antibiótico y monitorización de la vacuna.

En caso de que existiera alguna duda con posibles efectos tóxicos, tanto del tratamiento oral estándar como de la vacuna BCG, se procedería de acuerdo con los protocolos habituales de actuación en dichos casos.

4. Seguro

El promotor del estudio dispone de una póliza de seguros que se ajusta a la legislación vigente (**Real Decreto 1090/2015, de 4 de diciembre**, por el que se regulan los ensayos clínicos con medicamentos), que le proporcionará la compensación e indemnización correspondientes en caso de menoscabo de su salud o de lesiones que pudieran producirse en relación con su participación en el estudio.

Esta póliza está contratada con la compañía _____ con nº de póliza _____.

En caso de que se necesiten cuidados médicos, los gastos ocasionados por ello es responsabilidad del Promotor.

Le informamos que es posible que su participación en este ensayo clínico puede modificar las condiciones generales y particulares (cobertura) de sus pólizas de seguros (vida, salud, accidente…). Le recomendamos que se ponga en contacto con su compañía de seguros y le informe de su participación en este ensayo para determinar si esto podría afectar su póliza de seguro actual o alguna póliza nueva que vaya a contratar.

5. Número de urgencia para problemas en el ensayo

En caso de que desee formular preguntas acerca del estudio o daños relacionados con el mismo, contactar con el médico del estudio Dr. _____ en el número de teléfono _____.

6. Compensación económica

Los investigadores no obtendrán beneficio económico alguno procedente de la realización de este ensayo. Su participación en el mismo no le supondrá ningún gasto. Además, no tendrá que pagar por los medicamentos que le suministren en el estudio.

7. Información adicional

Cualquier nueva información referente a los fármacos utilizados en el estudio que se descubra durante su participación y que pueda afectar a su disposición para participar en el estudio, le será comunicada por su médico lo antes posible.

Si usted decide retirar el consentimiento para participar en este estudio, no se añadirá ningún dato nuevo a la base de datos y, puede exigir la destrucción de todas las muestras identificables previamente obtenidas para evitar la realización de nuevos análisis.

También debe saber que puede ser excluido del estudio si el promotor o los investigadores de este lo consideran oportuno, ya sea por motivos de seguridad, por cualquier acontecimiento adverso que se produzca por la mediación en estudio o porque consideren que usted no está cumpliendo con los procedimientos establecidos. En cualquiera de los casos, usted recibirá una explicación adecuada del motivo por el que se ha decidido su retirada del estudio.

El promotor podrá suspender el ensayo siempre y cuando sea por alguno de los supuestos contemplados en el Real Decreto.

Al firmar la hoja de consentimiento adjunta, se compromete a cumplir con los procedimientos del estudio que se le han expuesto.

Annex 3. Informed consent document

DOCUMENTO DE CONSENTIMIENTO INFORMADO PARA PARTICIPAR EN EL ENSAYO

Yo, _____, con DNI _____, declaro que:

He leído y comprendido la hoja informativa sobre el estudio que me han entregado, pudiendo realizar todas las cuestiones necesarias respecto al mismo.

Me han informado de las implicaciones y objetivos del estudio, así como de los posibles riesgos asociados a mi participación en él.

Entiendo que mi participación en el ensayo propuesto es voluntaria y no remunerada, y que puedo revocar el consentimiento sin necesidad de justificar las razones sin que esto suponga ninguna modificación en mi asistencia sanitaria. Entiendo que se respetará la confidencialidad de mis datos.

Me comprometo a cumplir con la adherencia al tratamiento oral pautado en mi domicilio tal y como se establece en el protocolo, así como a asistir a la administración de los fármacos endovenosos y a las sucesivas visitas de control que se van a realizar una vez al mes.

Deseo recibir información vía telefónica o por correo electrónico sobre los futuros resultados del estudio:

No ☐ Si ☐, en dicho caso indique	Correo electrónico: _____ Número de teléfono: _____

Por todo ello, otorgo mi consentimiento para participar en el ensayo y estoy de acuerdo en que la información obtenida en él pueda ser utilizada en investigaciones futuras sobre el manejo de la infección pulmonar por micobacterias no tuberculosas.

Firma del paciente Firma del investigador

Lugar y fecha: _____, ____ de _____ del 20____

Annex 4. Characteristics and safety of BCG (31)

Vaccine characteristics, content, dosage, administration, storage

BCG vaccines are usually administered by intradermal injection. Correct vaccine administration technique by a trained health worker is important to ensure correct dosage and optimal BCG vaccine efficacy and safety. B

BCG vaccination usually causes a scar at the site of injection due to local inflammatory processes. However, scar formation is not a marker for protection and approximately 10% of vaccine recipients do not develop a scar (63).

The standard dose of reconstituted vaccine is 0.05 mL for infants aged <1 year and 0.1 mL for infants aged >1 year. BCG vaccine is not available in combination with other vaccines.

The vaccine should not be exposed to direct sunlight or heat and should be stored at temperatures between 2 °C and 8 °C.

Immunogenicity, efficacy and effectiveness

In pulmonary TBC, effectiveness 0-80% depending on age groups mainly, but also depends on other factors. In case of Buruli ulcer (*M. Ulcerans*), was found to have ~50% efficacy (RR 0.5, 95% CI: 0.37–0.69) in African settings, is protective against NTM lymphadenitis in children.

Additional evidence comes from European countries which reported an increase in NTM infections after interrupting universal BCG vaccination.

Duration of protection and revaccination

Protection could last for up to 15 years in some populations but was found to decline with time. BCG-revaccination has been studied in the context of post-exposure prophylaxis for prevention of leprosy among contacts of cases, but these studies did not provide evidence of a benefit.

Safety

About 95% of BCG vaccine recipients experience a reaction at the injection site characterized by a papule which may progress to become ulcerated, with healing after 2–5 months leaving a superficial scar.

Adverse events following immunization (AEFI) with BCG are dependent on a number of factors including the strain used in the vaccine, number of viable bacilli in the batch, and variation

in injection technique. Severe AEFI include local reactions such as injection site abscess, severe ulceration or suppurative lymphadenitis usually caused by inadvertent injection of the vaccine sub-dermally.

BCG vaccine related complications may occur distal to the site of inoculation in the skin, intestines, bones (osteitis) or bone marrow (osteomyelitis) >12 months after vaccination. BCG immune reconstitution inflammatory syndrome (IRIS) also occurs in association with HIV infection. Other noted BCG syndromes have included uveitis and skin lesions such as lupus vulgaris.

Co-administration of vaccines: There is evidence that BCG vaccine can be safely co-administered with diphtheria-pertussis-tetanus (DTP), polio, hepatitis B, *Haemophilus influenzae type b* (Hib) and measles and rubella vaccines. There is no evidence to suggest reduced immunogenicity, and no safety concerns have been reported.

Special populations

Pregnant and lactancy: Although no harmful effects on the foetus have been observed, there is insufficient evidence about the safety of BCG vaccination during pregnancy. There is no contraindication for BCG vaccination of lactating women.

HIV-infected infants: Evidence shows that children who were HIV-infected at birth and vaccinated with BCG at birth, and who later developed AIDS, were at increased risk of developing disseminated BCG disease.

Preterm infants and low birth weight infants: Based on limited available evidence from small observational studies conducted in different high TB endemic settings, BCG vaccination at birth in healthy preterm infants born after 32–36 weeks of gestation was found to be safe and effective.

After vaccination

In general, this vaccination does not usually cause fever or discomfort.

A few days after vaccination an induration nodule develops at the injection site. This nodule gradually decreases and is replaced by a local lesion that can ulcerate a few weeks later. The local lesion does not require treatment. This lesion heals spontaneously.

Occasionally, thickening of the lymph, cervical or axillary nodules may be observed, which also does not require treatment.

Adverse events

Enlargement of lymph nodes (> 1 cm), headache, fever, ulcer at the injection site. Disseminated infection, such as acute or chronic inflammation of the bones, caused or not caused by an infection, inflammation with pus from the nodes, abscess in the injection area, allergic reaction, hypersensitivity reaction.

Overdose

An overdose increases the risk of suppurative lymphadenitis and can cause excessive formation of bedsores.

A massive overdose increases the risk of adverse effects of the BCG Vaccine.

Deep injection of the vaccine increases the risk of suppurating ulcer, lymphadenitis and abscess formation