A RANDOMIZED CLINICAL TRIAL FOR ENHANCED TRAINED IMMUNE RESPONSES THROUGH BACILLUS CALMETTE-GUÉRIN VACCINATION TO PREVENT INFECTIONS BY COVID-19: THE ACTIVATE II TRIAL

Running heading: BACILLUS CALMETTE-GUÉRIN VACCINATION TO PREVENT COVID-19

STUDY PROTOCOL

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EudraCT number: 2020-002448-21
Study phase: phase IV
Protocol version: 2.0
Protocol date: 18 June 2020

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DISCLOSURE OF PRINCIPAL INVESTIGATOR

Protocol Study Title: A RANDOMIZED CLINICAL TRIAL FOR ENHANCED TRAINED IMMUNE RESPONSES THROUGH BACILLUS CALMETTE-GUÉRIN VACCINATION TO PREVENT INFECTIONS BY COVID-19: THE ACTIVATE II TRIAL

The herein protocol became known to myself by the Study Sponsor. I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.

The Principal Investigator,

Print Name

Signature Date

The Sponsor,

Print Name

Signature Date

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LIST OF ABBREVIATIONS

AE: adverse event

BCG: Bacillus Calmette Guérin

CCI: Charlson's comorbidity index

CHD: coronary heart disease

COPD: chronic obstructive pulmonary disease

COVID-19: Coronavirus 2019 infection

HIV: human immunodeficiency virus

Ig: immunoglobulin

IGRA: interferon-gamma releasing assay

ml: microliter

PPD: skin tuberculin test

PBMCs: peripheral blood mononuclear cells

SAE: serious adverse event

SYNOPSIS

Aim	Based on findings of the interim analysis of the ACTIVATE study (EudraCT number, 2017-000596-87; ClinicalTrials.gov NCT03296423) showing 53% decrease of the incidence of all new infections with BCG vaccination, a new trial is designed aiming to validate If BCG can protect against COVID-19.
Design	Prospective, randomized, controlled trial
Inclusion criteria	1. Written informed consent 2. Male or female gender 3. Age more than or equal to 50 years based on the precise date of birth. Female participants are allowed on the premise that they are pros-menopausal. 4. History of at least one of the following: a) coronary heart disease; b) chronic obstructive pulmonary disease; c) Charlson's comorbidity index (CCI) more than 3 (APPENDIX III) 5. Negative serum testing for immunoglobulin G and M against SARS-CoV-2 6. Skin tuberculin test diameter less than 10mm
Exclusion criteria	• Deny to written informed consent • Age less than 50 years • Known infection by the Human Immunodeficiency Virus-1 (HIV-1) • Severely immunocompromised patients. This exclusion category comprises: ➢ History of congenital immunodeficiency ➢ History of solid organ transplantation ➢ History of bone marrow transplantation ➢ Intake of chemotherapy the last two months ➢ Intake of radiotherapy the last two months ➢ Active hemalogical or solid tumor malignancy ➢ History of any anti-cytokine therapies ➢ History of oral or intravenous steroids defined as daily doses of 10mg prednisone or equivalent for longer than 3 months

Study groups	• Placebo group: these patients will receive one intradermal injection of 0.1ml of sodium chloride 0.9% • BCG group: these patients will receive one intradermal injection of 0.1ml of BCG (Bacillus Calmette Guérin Moscow strain 361- I, Serum Institute for India)
Primary study endpoint	This is set on visit 3 (90 ± 5 days from the date of visit 1). The two groups of vaccination are compared for the primary endpoints which is composite. Patients who meet any of the following will be considered to meet the primary endpoint: • Positive for the respiratory questionnaire endpoint when at least one of the following combination is met either at visit 2 and/or at visit 3: ➢ One situation definitively related to COVID-19 ➢ All four questions of symptoms possibly related to COVID-19 answered YES ➢ At least two questions of symptoms possibly related to COVID-19 answered YES + need for admission at the emergency department of any hospital and/or need for intake of antibiotics answered YES ➢ At least four questions of symptoms probably related to COVID-19 answered YES one of which is “need for admission at the emergency department of any hospital and/or need for intake of antibiotics” • Positive IgG or IgM antibodies against SARS-CoV-2
Secondary study endpoints	• Positive respiratory questionnaire endpoint (as defined above) on visit 4 • Positive respiratory questionnaire endpoint (as defined above) on visit 5 • Prevalence of IgG/IgM against SARS-CoV-2 among the total of screened participants • Itemized analysis of each of the components of the respiratory questionnaire on each study visit • The impact of new cardiovascular events on each study visit • The differences in repeated measurements of vascular parameters

	(arterial hardness, central arterial pressures, reflected waves, endothelial function and thickness of medial carotid sheath) in the visit 3 between the two groups (placebo or BCG) • The differences in repeated measurements of vascular parameters (arterial hardness, central arterial pressures, reflected waves, endothelial function and thickness of the medial carotid sheath) and cardiac echocardiogram on the ECG visit (visit BCG) between the two sub-study groups • Changes in the release of cytokines from blood mononuclear cells at visit 3 between the two sub-study groups (placebo or BCG)
Power of the study	The study is powered for the primary endpoint. The results of the interim analysis of the ACTIVATE study reveal 20.4% incidence of total respiratory infections after three months from vaccination in the placebo group and 12.2% in the BCG group. To demonstrate this difference with 90% power at the 5% level of significance, 437 people need to be vaccinated per group. To adjust for losses to follow-up, we target to enroll 450 people per group. To estimate the power of the sub-study, it is anticipated that there will be a difference greater or equal to 2% in endothelial-dependent vasodilation between the two interventions in visit 5. It is estimated that a total sample of 68 patients (equally 34 individuals in each study group-placebo and BCG) will highlight the difference with 80% power at a statistical significance level of 10%. In order to adjust for possible loss of 10%, it is estimated that 75 patients should be admitted to the sub-study.
Interim analysis	Taking into consideration the situation of COVID-19 pandemic, an interim analysis is decided by the Sponsor when all patients reach visit 3
Study duration	12 months

BACKGROUND

Infection by the novel SARS-CoV-2 virus (also known as COVID-19) has tremendous social impact. Most of Western societies are at major or part lockdown whatever brings unpredictable financial and societal consequences. The urgent need for the reversal of this situation can only be met through the generation of an immune defence shield to protect the society from COVID-19. Many efforts for the development of a vaccine are under way without any specific outcome so for.

The stimulation of trained immune responses seems the only alternative to bridge the gap from the turn-on of the society until the entrance of a specific vaccine in the market. Trained immunity stands for the non-specific raise of defence shield for severe infections coming once tissue macrophages recognize a universal pathogen[1]. The concept was successfully tested in healthy volunteers that were vaccinated with placebo or BCG (Baccillus Calmette Guérin) vaccine. These volunteers were injected 14 days latter a tri-valent influenza A vaccine. Volunteers previously vaccinated by BCG developed significantly greater titles against hemagglutinin A of the influenza A virus whereas their circulating monocytes were more potent for the production of interferon-gamma[2].

It is proposed that this BCG vaccination triggering trained immune responses may play a role of protection against the COVID-19 pandemic[3]. A solid background on this rationale came recently from the interim analysis of the ACTIVATE trial. ACTIVATE (A randomized Clinical trial for enhanced Trained Immune responses through Bacillus Calmette-Guérin VAccination to prevenT infections of the Elderly) was a prospective randomized open-label controlled trial conducted among patients hospitalized at the 4th Department of Internal Medicine of ATTIKON University General Hospital in Greece. The protocol was approved by the National Ethics Committee of Greece and the National Organization for Medicine of Greece (EudraCT number, 2017-000596-87; ClinicalTrials.gov NCT03296423). The trial is conducted and funded by the Hellenic Institute for the Study of Sepsis. In this trial hospitalized elderly patients were vaccinated on the day of hospital discharge with single doses of placebo or BCG. Every patient is under follow-up for 12 months. The last visit of the last patient is scheduled for August 2020. An interim analysis took place on April 29th 2020 by an independent committee of experts. The full interim analysis (provided in APPENDIX I) focused on the study primary endpoint that was

the comparative time to a new infection between the two groups of treatment. Infections counting against this primary endpoint were respiratory or viral infections necessitating medical treatment, community-acquired pneumonias, hospital-acquired pneumonias, intraabdominal infections, urinary tract infections, soft tissue infections and bloodstream infections. Analysis revealed 53% decrease of the incidence of new infections in the BCG group compared to the placebo group. This decrease reached 80% for all respiratory tract infections. Multivariate analysis showed that most of benefit was for patients with coronary heart disease (CHD) and chronic obstructive pulmonary disease (COPD). This interim analysis clearly enhances the concept that BCG can be protective against COVID-19.

AIM OF THE STUDY

The aim of the study is to demonstrate in a double-blind, placebo-controlled approach if vaccination of participants susceptible to COVID-19 with BCG vaccine may modulate their disease susceptibility for COVID-19. This will be validated using both clinical and immunological criteria. A sub-study will run in order to assess the mechanism of benefit from BCG vaccination on vascular endothelial function and on mononuclear blood cells.

STUDY DESIGN

Patient population

This is a prospective, multicenter, randomized, controlled trial in that will take place in departments in Greece (APPENDIX II). The study will be submitted for approval by the National Ethics Committee of Greece and by the National Organization for Medicines of Greece. Patients will be enrolled after written informed consent provided by themselves or provided by legal representative in case of patients unable to consent.

Inclusion criteria

Enrolled patients should meet all following inclusion criteria:

1. Written informed consent
2. Male or female

3. Age more than or equal to 50 years based on the precise date of birth. Female participants are allowed on the premise that they are post-menopausal.
4. History of at least one of the following: a) coronary heart disease; b) chronic obstructive pulmonary disease; c) Charlson's comorbidity index (CCI) more than 3 (APPENDIX III)
5. Skin tuberculin test diameter less than 10mm
6. Negative serum testing for immunoglobulin G and M against SARS-CoV-2

Exclusion criteria

Patients meeting ANY of the following should NOT be enrolled.

- Deny to written informed consent
- Age less than 50 years
- Known infection by the Human Immunodeficiency Virus-1 (HIV-1)
- Severely immunocompromised patients. This exclusion category comprises:
  - History of congenital immunodeficiency
  - History of solid organ transplantation
  - History of bone marrow transplantation
  - Intake of chemotherapy the last two months
  - Intake of radiotherapy the last two months
  - Active hemalogical or solid tumor malignancy
  - History of any anti-cytokine therapies
  - History of oral or intravenous steroids defined as daily doses of 10mg prednisone or equivalent for longer than the last 3 months

Study interventions

Participants will be randomized at 1:1 ratio by a separate list per study site into two treatment groups. The list of randomization will be generated by a biostatistician and the randomization will be in a sealed envelope. The envelope will be delivered to the attending physicians after randomization. The two groups of treatment will be as follows:

- Placebo group: these participants will receive one intradermal injection of 0.1ml of sodium chloride 0.9%

- BCG group: these participants will receive one intradermal injection of 0.1ml of BCG (Bacillus Calmette Guérin Moscow strain 361- I, Serum Institute for India)

Patient screening and enrolment (APPENDIX IV)

In order for screening to proceed, the study will be advertised in the study site of the Hellenic Institute for the Study of Sepsis (www.sepsis.gr) and in the sites of other medical societies. The text to be used for the study advertisement is provided in APPENDIX V. People who will collaborate for the patient information will be GCP certified by the Sponsor.

Screening may start only after written informed consent is provided. Once written informed consent is provided, screening follows the following order:

- The patient is screened against the exclusion criteria. If the patient does not meet any exclusion criteria, screening proceeds to the next step.
- The patient is screened for inclusion criteria 1 to 4. If he does not meet all of them, it is a screening failure and screening stops. If he meets all of them, screening proceeds to the next step.
- The PPD Skin Test (inclusion criterion 5) is performed with an intradermal tuberculin injection into an forearm according to the manufacturer's instructions. The patient is asked to return in 48 hours to measure the skin hardness. If the diameter of the skin hardness is greater or equal to 10mm, it is considered as failure and screening process ends. If the diameter of the skin hardness is less than 10mm, screening process proceeds to the next step. This step can be omitted for patients who have undergone tuberculosis skin testing or a positive indirect detection test for *Mycobacterium tuberculosis* by determination of interferon-γ (IFNγ) after excitation of the last CD4- /CD8- cells for special peptides (IGRA) within the past five (5) years. In this case, any skin reaction with a fraction of less than 10 millimeters or a negative IGRA test allows the patient to enter the study.
- For patients who continue screening process, a diagnostic test using blood from the fingertip to detect IgG/IgM antibodies against the SARS-CoV-2 virus takes place (inclusion criterion 6). If the test is positive for any of IgM or IgG antibodies, screening stops. If the test for both IgG / IgM antibodies is negative, the patient may be included in the trial.

Sub-Study

Participants who will be included in the study in the Department of Clinical Therapeutics of the ALEXANDRA General Hospital of Athens, will participate in a sub-study at the Vascular Laboratory of the Therapeutic Clinic. The sub-study procedures are carried out within the framework of the planned visits of the patients and are analyzed in the paragraph “Patient visits and interventions”.

Study visits (APPENDIX IV)

Visit 1 This visit is conducted immediately after the end of the screening procedure (month 0 of study) and involves the following:

- Recording of the following information: age and gender, pre-existing comorbidities, history of hospitalizations the last two years
- For the participants of the sub-study, the following procedures will be performed: a) carotid and femoral artery ultrasound, b) heart ultrasound, and c) a blood sample of 10 ml (peripheral blood) will be collected for PBMC stimulation for cytokine production (see section "Sub-study procedures")
- Administration of placebo or BCG

Visit 2 This visit is conducted 45 ± 5 days from the date of visit 1. This visit may be done by phone call or through the internet. During this visit, the vaccinated subject is asked to provide answers to a specific questionnaire involving the incidence of respiratory tract infection (Appendix VI). All patients will also be asked for the incidence of cardiovascular events, such as any diagnosis of stroke, myocardial infarction or rupture of a abdominal aortic aneurysm.

Visit 3 This visit is conducted 90 ± 5 days from the date of visit 1. This is a visit conducted at the study site. During this visit, the following are done:

- IgG and IgM antibodies against SARS-CoV-2 are measured in the vaccinated subject in exactly the same method that was followed at the screening stage
- The vaccinated subject is asked to provide answers to a specific questionnaire involving the incidence of respiratory tract infection (Appendix VI). All patients will

be asked for the incidence of cardiovascular events, such as any diagnosis of stroke, myocardial infarction or rupture of a abdominal aortic aneurysm.

- For the participants of the sub-study, the following procedures will be performed: a) carotid and femoral artery ultrasound, b) heart ultrasound, and c) a blood sample of 10 ml (peripheral blood) will be collected for PBMC stimulation for cytokine production (see section "Sub-study procedures")

Visit 4 This visit is conducted 135 ± 5 days from the date of visit 1. This visit may be done by phone call or through the internet. During this visit, the vaccinated subject is asked to provide answers to a specific questionnaire involving the incidence of respiratory tract infection (Appendix VI). All patients will be asked for the incidence of cardiovascular events, such as any diagnosis of stroke, myocardial infarction or rupture of a abdominal aortic aneurysm.

Visit 5 This visit is conducted 180 ± 5 days from the date of visit 1. This visit may be done by phone call or through the internet. During this visit, the vaccinated subject is asked to provide answers to a specific questionnaire involving the incidence of respiratory tract infection (Appendix VI). All patients will be asked for the occurrence of cardiovascular events, such as any diagnosis of stroke, myocardial infarction or rupture of a abdominal aortic aneurysm. For the participants of the sub-study, the following procedures will be performed: a) carotid and femoral artery ultrasound, b) heart ultrasound, and c) a blood sample of 10 ml (peripheral blood) will be collected for PBMC stimulation for cytokine production (see section "Sub-study procedures")

It is explicitly stated that the investigators will encourage vaccinated population to refer to them in case of the incidence of any infection-related symptom.

PROCEDURES OF THE SUB-STUDY

Vaccinated people should abstain from food, alcohol and caffeine, smoking, and vasodilators for 12 hours before performing angiographic imaging studies. The exams to be taken are as follows:

- Arterial stiffness in the aorta from the speed of the pulse wave: It will be evaluated using a special non-invasive device (Complior, Artech Medical, France) in a non-bloody way on the carotid arteries and femoral arteries.
- Central blood pressure and reflected aortic waves: It will be evaluated using a special automated device (SphygmoCor System, AtCor Medical Pty Ltd, Sydney, Australia) in a non-bloody way on the carotid and femoral arteries.
- Endothelial function with ultrasound measurement of the dependent-from-the-endothelial dilation and mediated-from-the-nitrite dimension: It will be assessed using an ultrasound device and the measurement of the route-dependent vasodilator using a special 7-14 MHz audio recorder (Vivid 7 Pro, GE, USA) to be applied in a non-bloody way to the carotid and femoral arteries
- Carotid arteries intima-media thickness and Uterine Arteries: It will be evaluated using a B-mode ultrasound device and a special sound system (14.0 MHz multi-frequency linear array probe, Vivid 7 Pro, GE Healthcare, USA) in a non-bloody way on the carotid arteries. and femoral arteries.
- Cardiac ultrasound: The standard procedures of the clinical practice will be followed and the standard measurements will be taken using 2-D and Doppler ultrasound techniques.
- One blood sample will be taken from a forearm vein after antiseptic application and will be collected in tubes with anticoagulant agent. Isolation of PBMCs will be performed and they will be stimulated with purified bacterial antigens for cytokine production.

STUDY ENDPOINTS

Primary endpoint

The primary study endpoint is set on visit 3 (90 ± 5 days from the date of visit 1). The two groups of vaccination are compared for the primary endpoints which is composite. Patients who meet any of the following will be considered to meet the primary endpoint:

- Positive for the respiratory questionnaire endpoint when at least one of the following combination is met either at visit 2 and/or at visit 3 (see APPENDIX VI):
  - One situation definitively related to COVID-19

- All four questions of symptoms possibly related to COVID-19 answered YES
- At least two questions of symptoms possibly related to COVID-19 answered YES + need for admission at the emergency department of any hospital and/or need for intake of antibiotics answered YES
- At least four questions of symptoms probably related to COVID-19 answered YES one of which is “need for admission at the emergency department of any hospital and/or need for intake of antibiotics”

- Positive IgG or IgM antibodies against SARS-CoV-2

Secondary endpoints

The secondary endpoints are the difference between the two groups of vaccination in each of the following:

- Positive respiratory questionnaire endpoint (as defined above) on visit 4
- Positive respiratory questionnaire endpoint (as defined above) on visit 5
- Prevalence of IgG/IgM against SARS-CoV-2 among the total of screened participants
- Itemized analysis of each of the components of the respiratory questionnaire on each study visit
- The incidence of new cardiovascular events on each study visit
- Differences in repeated measurements of vascular parameters (arterial hardness, central arterial pressures, reflected waves, endothelial function and carotid medial sheath thickness) in visit 3 between the two sub-study groups (placebo or BCG)
- Differences in repeated measurements of vascular parameters (arterial hardness, central arterial pressures, reflected waves, endothelial function and thickness of medial carotid sheath) and cardiac ultrasound at visit 5 between the two sub-study groups (placebo or BCG)
- Changes in the release of cytokines from blood mononuclear cells at visit 3 between the two sub-study groups (placebo or BCG)

STUDY POWER CALCULATION

The study is powered for the primary endpoint. The results of the interim analysis of the ACTIVATE study reveal 20.4% incidence of total respiratory infections

after three months from vaccination in the placebo group and 12.2% in the BCG group. To demonstrate this difference with 90% power at the 5% level of significance, 437 people need to be vaccinated per group. To adjust for losses to follow-up, we target to enroll 450 people per group.

To estimate the power of the sub-study, it is anticipated that there will be a difference greater or equal to 2% in endothelial-dependent vasodilation between the two interventions in visit 5. A total sample of 68 patients (with equally 34 individuals in each study group placebo or BCG) will highlight a difference with a power of 80% at the 10% level of significance. In order to adjust for possible losses of 10%, it is estimated that 75 patients should be admitted to the sub-study.

STATISTICAL ANALYSIS

Comparisons between the two groups will be done by the Fisher exact test. Any value of p below 0.05 will be considered as significant.

The differences in the repeated measurements of the vascular parameters (arterial hardness, central arterial pressures, reflected waves, endothelial function and thickness of the inner-middle carotid sheath) will be estimated using randomly mixed models with two types: random effects (random intercept and random slope) and variance-covariance matrix. Vascular parameters will be used as dependent variables, after checking the normal distribution of their residuals with histograms and Q-Q plots graphs. In case of irregular distribution, appropriate transformations will be used (based on the neural index or using inverse ranking normalization).

INTERIM ANALYSIS

The Sponsor will appoint an independent committee to analyze the study and report the results of the primary endpoint once the last enrolled subject will reach visit 3. The study investigators will be informed on the results of the interim analysis but they will remain blind to the allocated arm of vaccination which will allow the study to end after the last visit of the last subject.

ADVERSE EVENTS

Adverse events (AEs) and Serious Adverse Events (SAEs) will be collected from baseline until the last patient's evaluation. Investigators should monitor subjects for adverse events and are responsible for recording ALL adverse events and serious adverse events occurring to a patient during the trial. Infection will not be reported as an SAE since this is the study primary endpoint.

An adverse event is any undesirable medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The time relationship is defined from the moment the AE occurs during therapeutic treatment until 30 days or 5 half-lives after treatment discontinuation. The adverse event may be a sign, a symptom, or an abnormal laboratory finding.

Serious adverse events (SAEs) must be reported to within 24 hours. If an adverse event meets any of the following criteria, it is considered SAE:

- **Death**
- **Life-threatening situation** The subject was at risk of death at the time of the adverse event/experience. It does not refer to the hypothetical risk of death if the AE were more severe or were to progress.
- **Inpatient hospitalization** or prolongation of existing hospitalization.
- **Persistent or significant disability/incapacity** Any AE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions, including the ability to work. This is not intended to include transient interruption of daily activities.
- **Congenital anomaly/birth defects** Any structural abnormality in subject's offspring that occurs after intrauterine exposure to treatment.
- **Important medical events/experiences** that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event/experience when, based upon appropriate medical judgment, **they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above,** i.e., death, a life-threatening adverse event/experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Examples of such medical events/experiences include

allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

- **Spontaneous and elective abortions** experienced by study subject.

A non-serious adverse event is any untoward medical occurrence in a patient or subject who is administered a pharmaceutical product, and which does not necessarily have a causal relationship with this treatment. A non-serious adverse event is one that does not meet the definition of a serious adverse event given.

Grading of severity

The severity of the adverse events shall be graded as:

- **Mild** the adverse event is transient and well tolerated by the patient
- **Moderate** the adverse events causes discomfort and affects the usual activities of the patient.
- **Severe** the adverse events affects the usual activities of the patient to an important degree and may cause disability or be life-threatening.

Relationship to the drug

The investigator will use the following definitions to assess the relationship of the adverse event to study drug:

- **Probably Related**: The adverse event has a strong time relationship to the drug or relapses if re-induced, and another etiology is improbable or clearly less probable.
- **Possibly Related**: The adverse event has a strong time relationship to the drug and an alternative aetiology is as probable or less probable.
- **Probably not Related**: The adverse event has a slight or no time relationship to the drug and/or there is a more probable alternative aetiology.
- **Unrelated**: The adverse event is due to an underlying or concomitant disease or to another pharmaceutical product and is not related to the drug (no time relationship and a much more probable alternative aetiology).

If an investigator's opinion of possibly related, probably not related or not related to study drug is given, an alternate etiology must be provided by the investigator. Please note that a severe adverse event/experience is not necessarily serious, as the term severe is a measure of intensity while a serious adverse event is determined based on the aforementioned regulatory criteria. Individual un-blinding

thought to be necessary for the management of an adverse event will be documented in the subject Case Report Form.

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APPENDIX I Interim analysis of the ACTIVATE trial

A RANDOMIZED CLINICAL TRIAL FOR ENHANCED TRAINED IMMUNE RESPONSES THROUGH BACILLUS CALMETTE-GUÉRIN VACCINATION TO PREVENT INFECTIONS OF THE ELDERLY: THE ACTIVATE TRIAL

INTERIM ANALYSIS

By the board of April 29th 2020

Participants:

Prof. Jos W. M. van der Meer, chairing

Prof. Christine Stabell Benn

Miltiades Kyprianou

ACTIVATE was a prospective randomized open-label controlled trial among patients hospitalized at the 4th Department of Internal Medicine of ATTIKON University General Hospital in Greece. The protocol and its subsequent amendments were approved by the National Institutional Review Board and the National Ethics Committee of Greece, and by the Ethics Committees of ATTIKON hospital (EudraCT number, 2017-000596-87; ClinicalTrials.gov NCT03296423).

The study enrolled elderly patients (age ≥ 65 years) of both genders who were discharged from hospital after hospitalization for a medical cause. The main exclusion criteria were: a) solid organ malignancy or lymphoma diagnosed the last five years; b) treatment with oral or intravenous steroids defined as daily doses of 10mg prednisone or equivalent for longer than 3 months; c) severe immunodeficiency like infection by the human immunodeficiency virus, neutropenia, transplantation, intake of chemotherapy, primary immunodeficiency, severe and treatment with anti-cytokine therapies; and d) positive Interferon-gamma Release Assay (IGRA). All patients or their legal representatives provided written informed consent before enrollment.

On the day of hospital discharge and after careful recording of detailed medical history and laboratory examinations for the inclusion and exclusion criteria, patients remaining eligible underwent IGRA test. Those who had negative IGRA were allowed to be enrolled in the study. Participants were randomized to one intradermal vaccination 0.1ml of sodium chloride 0.9% or with 0.1ml of BCG (BCG vaccine Bulgaria strain 1331; Intervax). Randomization was performed in a 1:1 ratio by a biostatistician and was kept in a sealed envelope, which was delivered to the investigators for treatment allocation at randomization.

The primary outcome was the time interval to the first infection post hospital discharge between the two groups of treatment. This was a composite endpoint involving any of the following infections: respiratory tract infections of probable viral origin necessitating medical attention; bacteremia; community-acquired pneumonia; hospital-acquired pneumonia; urinary tract infection; intraabdominal infection; and soft-tissue infection. The definitions of these infections are provided in the Supplementary Appendix.

Secondary study outcomes included the rate of hospitalizations until month 12; the time to first sepsis episode; the total number of infections; the time to first hospitalization; the number of antibiotic courses; and one-year mortality. Sepsis was

defined by the Sepsis-3 definitions, i.e., sequential organ failure assessment (SOFA) score of ≥2 points for emergency admission patients and a ≥2-point increase on the admission SOFA score for hospitalized patients.

Patients' visits were conducted every month for 12 months. During each visit the following data were captured: history of any new infection; any new hospitalization followed by thorough study of hospital discharge notes and contact with treating physicians if necessary; any need for antimicrobial prescription; and patient disposition. All patients were monitored throughout the study period for adverse events and serious adverse events.

The sample size was calculated assuming the median time to new infection would be 4 months in the placebo group and 7 months with BCG vaccination. To achieve so with 90% power at the 5% level of significance, 100 patients were allocated to each arm. The interim analysis included only patients with completed 12-month of follow-up. In order to preserve the overall Type I error rate at 5 %, an adjustment of the level of significance of the interim and final analysis was done by O'Brien-Fleming strict alpha adjustment[1]. This adjustment provides significance a=0.0054 at interim and a=0.0492 at final. Time-to-event comparisons were done by the log-rank test and Cox regression reporting the hazard ratio (HR) with 95% confidence intervals (CI); quantitative data were compared by the Fisher's exact test. and by forward step-wise logistic regression analysis. A sensitivity analysis was conducted to assess robustness, in which the entire number of participants (198) was entered. Analysis was conducted using IBM SPSS Statistics v. 26.0. All p-values were two-sided and any p-value <0.05 was considered as statistically significant. The complete statistical analysis plan is provided in the protocol.

RESULTS

Patients

From September 2017 through August 2019, 202 patients were enrolled and randomized; four patients withdrew consent and requested removal of all data, leaving a final intention-to-treat analysis cohort of 198 patients. No patient was reported as lost to follow-up (Figure 1). Interim analysis included 78 patients allocated to placebo vaccination and 72 patients allocated to BCG vaccination. Baseline characteristics were similar between the two arms (Tables 1 and 2).

Primary and secondary outcomes

The primary outcome of the time until a new infection was significantly prolonged in the placebo vaccination group compared to the BCG vaccination group (P=0.04) (Figure 2). The hazard ratio of 0.53 provides indication that the rate of infection was almost twofold in the placebo group in comparison to the BCG group. The incidence of new infection was 37.2% (95% CIs 27.3-48.3%) in the placebo group and 20.8% (95% CIs in the BCG group (13.0-35.6%) in the BCG group with an odds ratio of 0.44 (Table 3). It is interesting to note that the main differences between the two groups involved infections of the respiratory system (Respiratory or viral infection necessitating medical treatment, community-acquired pneumonia and hospital-acquired pneumonia), where the odds ratio is reduced to 0.20 (Table 3 and Figure 3).

Stepwise logistic regression analysis showed that BCG vaccination was an independent protective factor from the incidence of new infection until month 12 (odds ratio, 0.44; 95% CI, 0.21 to 0.94; P=0.03) (Table 4). Sensitivity analysis including the total sample confirmed the findings (Table 5).

Major benefit from BCG vaccination was observed in one main secondary endpoint: mean number of infections until month 12 being 0.44 in the placebo group and 0.25 in the BCG group (P= 0.03) (Table 3). No difference in the other secondary endpoints was found between the two groups (Table 3).

A trend for lower serious adverse event was recorded in the BCG vaccination group than in the placebo group (Table 6). Moreover, the incidence of non-serious adverse events did not differ between the two groups. None of the adverse events were related to the study.

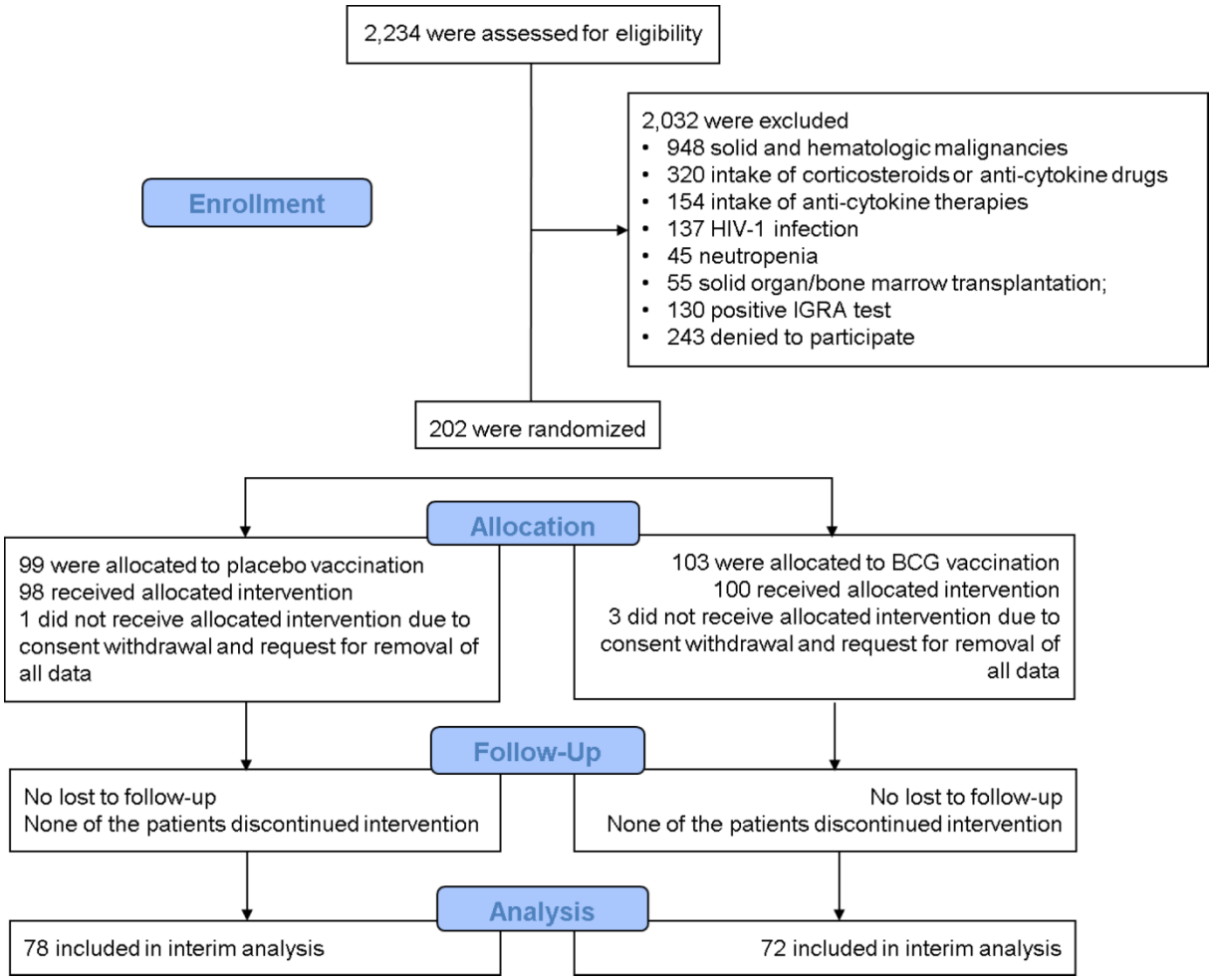

Figure 1. Screening, Randomization, and Follow-up.

Abbreviations: BCG: Bacillus Calmette-Guérin; HIV human immunodeficiency virus; IGRA: interferon-gamma releasing assay

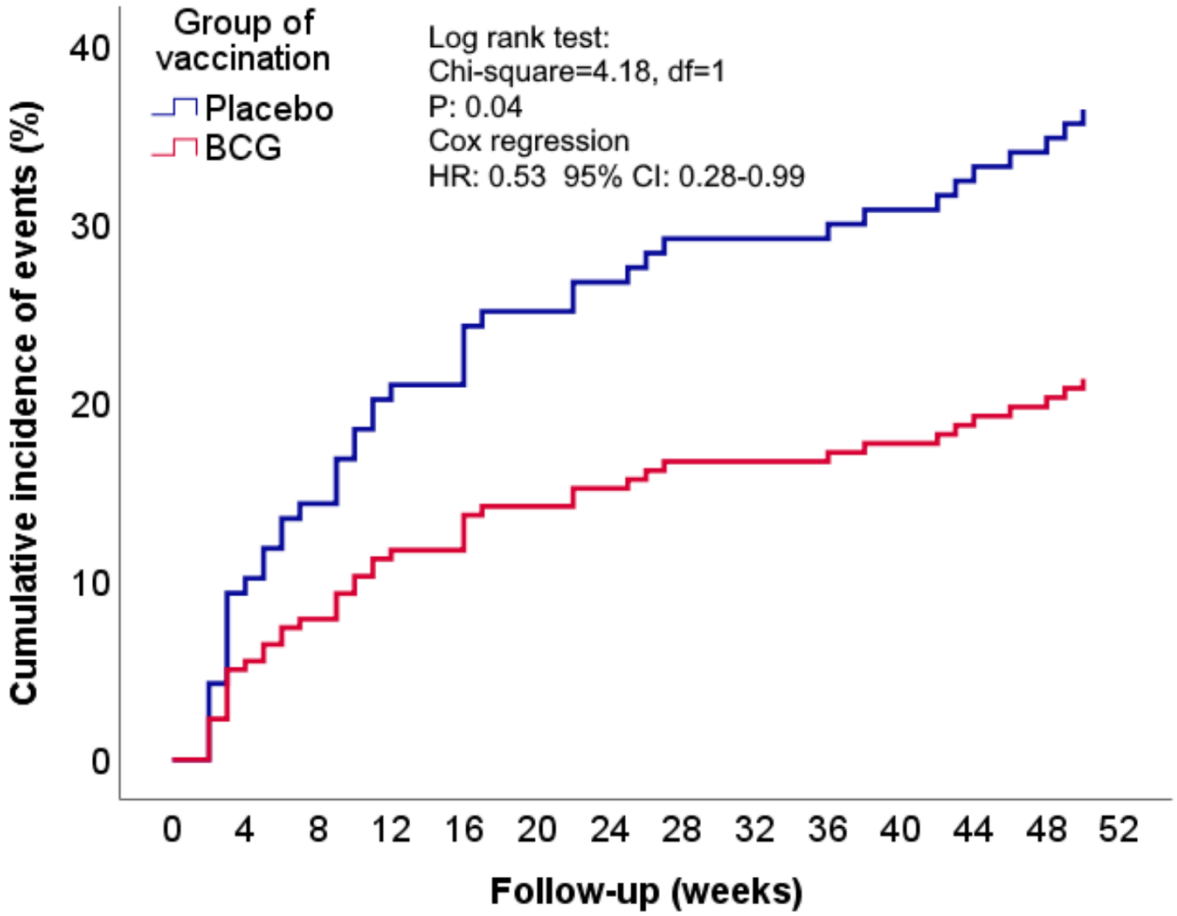

Figure 2. Cumulative Incidence of the Event of First Infection in the Two Groups of Vaccination

Infections counting against the primary endpoint were respiratory infection of probable viral origin necessitating medical attention, community-acquired pneumonia, hospital-acquired pneumonia, intraabdominal infections, urinary tract infection, bloodstream infections and acute bacterial skin and skin structure infections

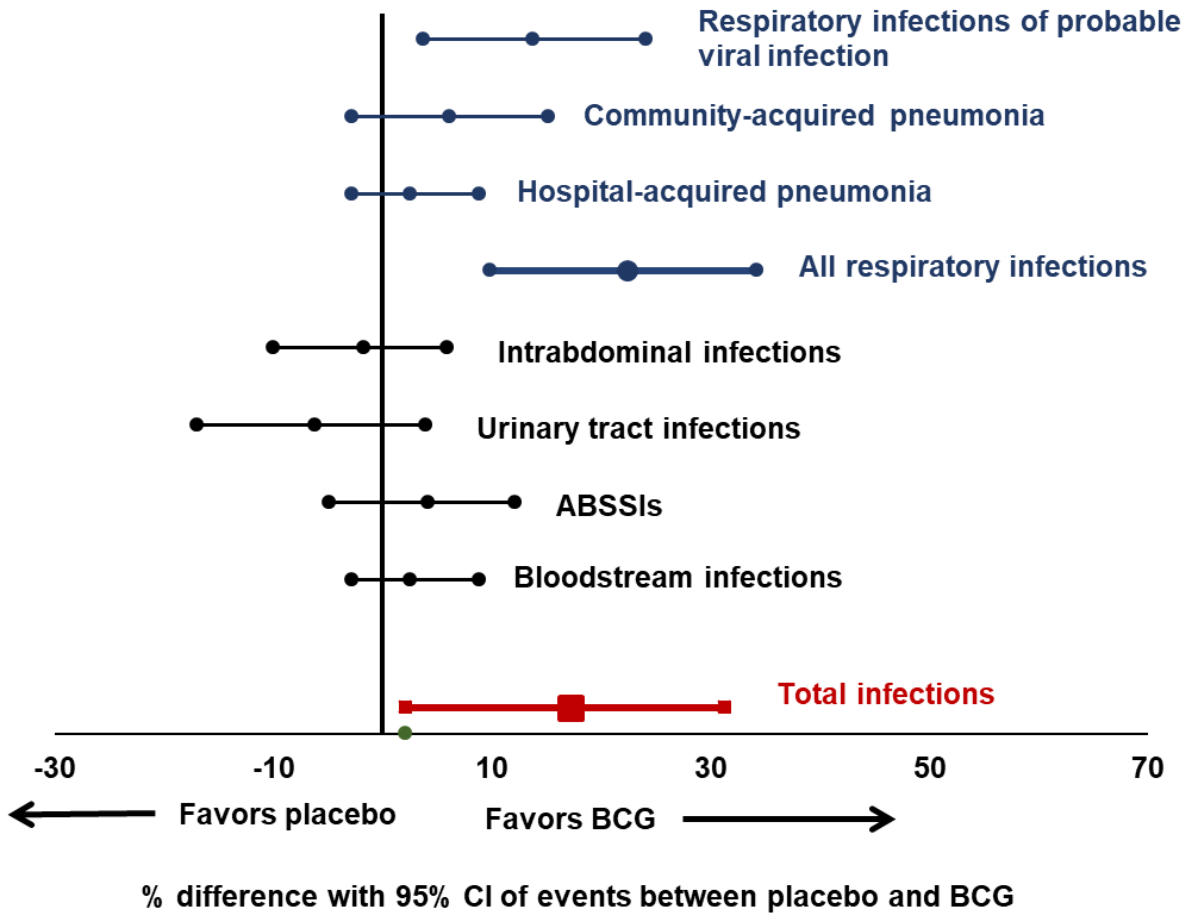

Figure 3 % difference of the incidence of each infection and 95% confidence intervals between the two groups

Table 1. Baseline Characteristics of Enrolled Patients.

	Placebo (N=78)	BCG (N=72)	p-value
Age (years), mean (SD)	79.6 (7.8)	79.9 (7.6)	0.80
Male gender — no. (%)	35 (44.9)	32 (44.4)	1.00
CCI, mean (SD)	5.5 (1.9)	5.5 (2.2)	0.91
APACHE II score on hospital discharge, mean (SD)	7.9 (3.0)	8.1 (2.9)	0.70
SOFA score on hospital discharge, mean (SD)	1.2 (1.4)	1.0 (1.1)	0.59
Comorbidities (%)			
Diabetes mellitus — no. (%)	29 (37.2)	23 (31.9)	0.61
without organ damage — no. (%)	23 (29.5)	15 (20.9)	0.26
with organ damage — no. (%)	6 (7.7)	8 (11.1)	0.58
Chronic heart failure — no. (%)	23 (29.5)	20 (27.8)	0.86
Chronic renal disease — no. (%)	14 (17.9)	12 (16.7)	1.00
Chronic obstructive pulmonary disease — no. (%)	12 (15.4)	11 (15.3)	1.00
Cerebrovascular disease — no. (%)	17 (21.8)	21 (29.2)	0.35
Degenerative disease — no. (%)	8 (10.3)	6 (8.3)	0.78
Myocardial infarct — no. (%)	13 (16.7)	9 (12.5)	0.49
Biliary stones — no. (%)	10 (12.8)	11 (15.3)	0.81
Renal stones — no. (%)	1 (1.3)	1 (1.4)	1.00
Any surgery — no. (%)	30 (38.5)	30 (41.7)	0.74
Dementia — no. (%)	15 (19.2)	20 (27.8)	0.25
Hemiplegia — no. (%)	1 (1.3)	1 (1.4)	1.00
Peptic ulcer disease — no. (%)	3 (3.8)	3 (4.2)	1.00
Peripheral vascular disease — no. (%)	1 (1.3)	0 (0)	1.00

Liver disease — no. (%)	1 (1.3)	1 (1.4)	1.00
Hypertension — no. (%)	56 (71.8)	53 (73.6)	0.86
Atrial fibrillation — no. (%)	30 (38.5)	22 (30.6)	0.39

Abbreviations: APACHE Acute physiology and chronic health evaluation; CCI: Charlson's Comorbidity Index; SD standard deviation; SOFA sequential organ failure assessment.

Table 2. Causes of hospital admission before study enrolment

Cause of hospital admission	Placebo (N=78)	BCG (N=72)	p-value
Lower respiratory tract infection — no. (%)	20 (25.6)	23 (31.9)	0.47
Biliary tract infection — no. (%)	8 (10.3)	7 (9.7)	1.00
Ischemic stroke — no. (%)	10 (12.8)	12 (16.7)	0.65
Acute kidney injury — no. (%)	4 (5.1)	4 (5.6)	1.00
Gastrointestinal tract bleeding — no. (%)	8 (10.3)	6 (8.3)	0.78
Anemia — no. (%)	4 (5.1)	2 (2.8)	0.68
Electrolyte disturbance — no. (%)	2 (2.5)	4 (5.6)	0.43
Pulmonary embolism — no. (%)	2 (2.6)	2 (2.8)	1.00
Cerebral hemorrhage — no. (%)	1 (1.3)	1 (1.4)	1.00
Acute pyelonephritis — no. (%)	6 (7.7)	2 (2.8)	0.28
ABSSI — no. (%)	4 (5.1)	0 (0)	0.12
Other disease — no. (%)	9 (11.5)	8 (11.1)	1.00

Abbreviation: ABSSSI: acute bacterial skin and skin structure infection

Table 3. Primary and Secondary Study Outcomes.

	Placebo (N=78)	**BCG (N=72)**	**Odds Ratio (95% CI)**	**p-value**
Patients with at least one new infection until month 12 — no. (%)*	**33 (42.3)**	**18 (25.0)**	**0.46 (0.23-0.91)**	**0.04**
Respiratory or viral infection necessitating medical treatment— no. (%)	14 (17.9)	3 (4.2)	0.20 (0.06-0.72)	0.01
Community-acquired pneumonia — no. (%)	8 (10.3)	3 (4.2)	0.38 (0.10-1.49)	0.21
Hospital-acquired pneumonia — no. (%)	2 (2.6)	0 (0)	-	0.50
Total respiratory infections — no. (%)	24 (30.1)	6 (8.3)	0.20 (0.08-0.54)	0.00
Intrabdominal infection — no. (%)	3 (3.8)	4 (5.6)	1.47 (0.32-6.81)	0.71
Urinary tract infection — no. (%)	6 (7.7)	8 (11.1)	1.5 (0.49-4.56)	0.58
Acute bacterial skin and skin structure infection — no. (%)	6 (7.7)	3 (4.2)	0.52 (0.13-2.17)	0.50
Bloodstream infection — no. (%)	2 (2.6)	0 (0)	-	0.49
Mean rate of hospitalization/patient until month 12 (SD)	0.49 (0.72)	0.43 (0.72)	N/A	**0.63**
Mean number of infections until month 12 (SD)	0.44 (0.61)	0.25 (0.52)	N/A	**0.03**
Mean courses of antibiotics until month 12 (SD)	0.69 (1.66)	0.60 (1.25)	N/A	**0.44**

One-year mortality — no. (%)	14 (17.9)	10 (13.9)	0.74 (0.31-1.78)	**0.51**

*some patients had more than one infection

Abbreviations: CI confidence interval; N/A not applicable; SD; standard deviation. * - differences in proportion with 95% CI, shown as percentages.

Table 4. Univariate and multivariate analysis of the effects of covariates on the incidence of at least one infection until month 12

	Univariate analysis			Multivariate analysis		
Covariates	OR	95% CI	p-value	OR	95% CI	p-value
Group of vaccination	0.46	0.23-0.91	0.03	0.43	0.21-0.88	0.02
Myocardial infarct	2.71	1.08-6.79	0.03	-	-	0.06
COPD	3.0	1.21-7.46	0.02	3.14	1.24-7.95	0.02

* Covariates that failed to enter the stepwise logistic regression model

Table 5. Sensitivity analysis of the incidence of new infections

	Placebo n/ total (%)	BCG n/total (%)	Difference in proportion % (95% CI)	OR	95% CI	p-value
Interim (150)	33/78 (42.3)	18/72 (25.0)	17.3 (2.1-31.3)	0.46	0.23-0.91	0.04
Total (198)	41/98 (41.8)	27/100 (27.0)	14.8 (1.6-27.4)	0.51	0.28-0.93	0.04

Abbreviations: CI: confidence interval; OR: odds ratio

Table 6. Adverse Events (AEs)

Serious AEs	**Placebo (N=78)**	**BCG (N=72)**	**p-value**
Presence of at least one SAE* — no. (%)	30 (38.5)	17 (23.6)	0.05
Death* — no. (%)	8 (10.3)	5 (6.9)	0.57
SAEs with hospitalization* — no. (%)	20 (25.6)	10 (13.9)	0.10
Reason for hospitalization — no. (%)			
Arrythmia	1 (1.3)	0 (0)	1.00
Stroke	2 (2.6)	1 (1.4)	1.00
Acute kidney injury	0 (0)	1 (1.4)	0.48
Deep vein thrombosis	1 (1.3)	0 (0)	1.00
Epilepsy	1 (1.3)	0 (0)	1.00
Electrolyte disturbance	1 (1.3)	0 (0)	1.00
Pulmonary edema	1 (1.3)	0 (0)	1.00
Anemia	1 (1.3)	0 (0)	1.00
ST-segment elevation at ECG	1 (1.3)	0 (0)	1.00
Elective surgery	2 (2.6)	2 (2.8)	1.00
SAEs without hospitalization — no. (%)			
Stroke — no. (%)	1 (1.3)	0 (0)	1.00
Syncope	0 (0)	1 (1.4)	0.48
Anemia	1 (1.3)	0 (0)	1.00

* - SAEs and deaths due to infections counting against the primary endpoint are not encountered here since per protocol they should not be reported as adverse events

Non-serious (AEs)			
At least one non-serious AE — no. (%)	20 (25.6)	26 (36.1)	0.21

Type of non-serious AE — no. (%)			
Varicella-zoster eruption	1 (1.3%)	0 (0)	1.00
Helicobacter pylori infection	3 (3.8)	0 (0)	0.24
Dacryocystitis	0 (0)	1 (1.4)	0.48
Hip fracture	2 (2.6)	0 (0)	0.49
Non-infection associated cough	4 (5.1)	11 (15.3)	0.06
Asymptomatic bacteriuria	2 (2.6)	7 (9.7)	0.09
Breast cancer	1 (1.3)	1 (1.4)	1.00
Renal cancer	0 (0)	1 (1.4)	0.48
Squamous skin carcinoma	0 (0)	1 (1.4)	0.48
Rash at the injection site	0 (0)	2 (2.8)	0.22
Otitis	0 (0)	1 (1.4)	0.48
Dental infection	2 (2.6)	1 (1.4)	1.00

References

1. DeMets DL, Lan KKG. Interim analysis: the alpha spending function approach. Statistics in Medicine. 1994;13:1341–1352.

APPENDIX II List of study sites

- 4th Department of Internal Medicine, ATTIKON University General Hospital (PI: Associate Professor A. Papadopoulos)
- 2nd Department of Internal Medicine, Ippokrateion General Hospital of Athens (PI: Assistant Prof. Helen Sambatakou)
- 3rd Department of Internal Medicine, Sotiria General Hospital of Athens (PI: Assistant Professor Garyfalia Poulakou)
- 1st Department of Internal Medicine, Athens General Hospital “G.Gennimatas” (PI: Dr. George Adamis)
- Department of Clinical Therapeutics, ALEXANDRA General Hospital of Athens (PI: Associate Professor Kimon Stamatelopoulos)
- 1st Department of Internal Medicine, AHEPA General Hospital of Thessaloniki (PI: Associate Professor Symeon Metallidis)
- Department of Internal Medicine, Patras University General Hospital, Rion (PI: Professor Charalambos Gogos)
- 2nd Department of Internal Medicine, Ioannina University General Hospital, Ioannina (PI: Professor Charalambos Milionis)
- 1st Department of Internal Medicine, Alexandroupolis General Hospital (PI: Assistant Professor Periklis Panagopoulos)
- Department of Internal Medicine, General Hospital of Ptolemaida (PI: Konstantinos Dolianitis)
- Department of Internal Medicine, General Hospital of Imathia, Veroia Unit (PI: Dr. Christos Koutras)
- Department of Surgery, General Hospital of Nafplion (PI: Dr. K.Katsaros)
- Department of Cardiology, Korinthos General Hospital (PI: Dr. Christos Hasikidis)
- Department of Pulmonary Medicine, General Hospital of Kerkyra (PI: Dr Ilias Papanikolaou)
- Department of Internal Medicine, General Hospital of Karditsa (PI: Dr Ioannis Ntelis)

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APPENDIX III The Charlson's comorbidity index (CCI)

CCI is defined after adding the score for age to the score for co-morbidities.

The score for age is given below:

Age group (years)	Points
0-49	0
50-59	1
60-69	2
70-79	3
80-89	4
90-99	5

The score for comorbidities is given below:

Condition	Points
None	0
• Myocardial infarct	1

(one or more definite or probable myocardial infarctions)

- Congestive heart failure

(exertional or paroxysmal nocturnal dyspnea treated with digitalis, diuretics, or afterload reducing agents)

- Peripheral vascular disease

(intermittent claudication or bypass for arterial insufficiency or gangrene or acute arterial insufficiency or an untreated thoracic or abdominal aneurysm ≥6 cm)

- Cerebrovascular disease (except hemiplegia)

(history of a cerebrovascular accident with minor or no residua and transient ischemic attacks)

- Dementia (defined as chronic cognitive deficit)
- Chronic pulmonary disease

• Connective tissue disease (systemic lupus erythematous, polvmyositis. mixed connective tissue disease, polymyalgia rheumatic and moderate to severe rheumatoid arthritis) • Peptic ulcer disease • Mild liver disease (cirrhosis without portal hypertension or chronic hepatitis) • Diabetes (without complications)	
• Diabetes with end organ damage (Retinopathy, neuropathy, nephropathy, previous hospitalizations for ketoacidosis, hyperosmolar coma, or juvenile onset or brittle diabetics) • Hemiplegia • Moderate or severe renal disease (moderate defined as serum creatinine of 2-3 mg/dl, severe defined as need for dialysis/transplantation) • Solid tumor (non-metastatic) (without documented metastases, but initially treated in the last five years) • Leukemia/lymphoma/multiple myeloma	2
• Moderate or severe liver disease (Moderate: cirrhosis with portal hypertension; severe: cirrhosis with portal hypertension and a history of variceal bleeding	3
• Metastatic solid tumor • AIDS (acquired immunodeficiency syndrome) (Definite or probable AIDS)	6

APPENDIX IV Study visits

Visit	**Screening**	**1**	**2**	**3**	**4**	**5**
Day		**0**	**45 ± 5**	**90 ± 5**	**135 ± 5**	**180 ± 5**
Informed consent	x					
Exclusion criteria	x					
Inclusion criteria	x					
IgG/IgM	x			x		
PPD	x					
Comorbidities	x	x				
Recent hospitalizations		x				
Vaccination		x				
Questionnaire		x	x	x	x	x
Cardiovascular events		x	x	x	x	x
Blood sampling*		x		x		x
Vascular ultrasound*		x		x		x
Heart ultrasound*		x		x		x

*only for participants in the sub-study

APPENDIX V Advertisement text

Recent data have shown that vaccination with BCG (this is the name of the vaccine for tuberculosis) offers a type of “defense shield” against various bugs and viruses. More precisely, the data showed that vaccination with BCG decreased the changes of getting one new respiratory infection by 80%. This generates hope that BCG may protect, at least transiently, from COVID-19.
The Hellenic Institute for the Study of Sepsis is coordinating a large-scale vaccination program to study if BCG can protect from COVID-19. This program is licensed by the Greek regulatory authority. People who are eligible for vaccination should have the following characteristics:

- Male or female aged ≥50 years
- Suffer from coronary heart disease or chronic obstructive pulmonary disease or several comorbidities

Patients who are interested to receive vaccination should visit one of the 12 departments in Greece where the program is taking place. Once they arrive at the department, they will be subject to examination by a team of physicians. Based on the results of this examination they will learn if they may participate or not in the vaccination program. Those who are interested to participate in the program may contact the following persons to get information on the most appropriate department to visit and on the exact procedure to be followed:

- Aggeliki Konstantopoulou, (tel, e-mail)
- Elena Konstantopoulou, (tel, e-mail)
- Varvara Perraki (tel; e-mail)
- Danai Prasianaki (tel:. e-mail)

The participating departments are in:

- Athens (ATTIKON hospital, Ippokrateion hospital, “G.Gennimatas” General Hospital, Sotiria hospital, General Hospital of Athens “Alexandra”)
- Thessaloniki (AHEPA hospital)
- Alexandroupolis (University hospital)
- Ioannina (University hospital)

- Veria (General hospital)
- Patras (Rion University hospital)
- Ptolemaida (General hospital)
- Nafplion (General hospital)
- Korinthos (General hospital)
- Kerkyra (General hospital)
- Karditsa (General hospital)

APPENDIX VI Questionnaire addressed to patients on study visits

The questionnaire is composed of questions referring to symptoms possible related to COVID-19, probably related to COVID-19 and definitively related to COVID-19.

Did you present any of the following symptoms during the last 45 days (please answer with YES or NO to any of the following infections):	**YES**	**NO**
Symptoms possibly related to COVID-19		
• Cough for more than 48 hours without any other symptoms		
• Shortness of breath for more than 48 hours without any other symptoms		
• Fever above >38°C for more than 48 hours without any other symptoms		
• Expectoration for more than 48 hours without any other symptoms		
Symptoms probably related to COVID-19		
• Cough and shortness of breath for more than 48 hours		
• Cough and fever for more than 48 hours		
• Fever and shortness of breath for more than 48 hours		
• Fever and expectoration for more than 48 hours		
• Need for admission at the emergency department of any hospital		
• Need for intake of antibiotics		
Situation definitively related to COVID-19		
• Had known diagnosis of COVID-19 by molecular test		

Patients score positive for the respiratory questionnaire endpoint if they have one of the following combination:

- One situation definitively related to COVID-19
- All four questions of symptoms possibly related to COVID-19 answered YES

- At least two questions of symptoms possibly related to COVID-19 answered YES + need for admission at the emergency department of any hospital and/or need for intake of antibiotics answered YES
- At least four questions of symptoms probably related to COVID-19 answered YES one of which is “need for admission at the emergency department of any hospital and/or need for intake of antibiotics”