Using BCG vaccine to enhance non-specific protection of senior citizens during the COVID-19 pandemic. A randomized clinical trial.

(BCG-DENMARK-SENIOR)

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Protocol title	**Using BCG vaccine to enhance non-specific protection of senior citizens during the COVID-19 pandemic. A randomized clinical trial.**
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
AR	Adverse Reaction
BCG	Bacillus Calmette-Guérin
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects
COVID-19	Coronavirus Disease, i.e., the disease caused by SARS-CoV-2.
CV	Curriculum Vitae
DKMA	Danish Medicines Agency
EC	The regional Committees on Health Research Ethics for Southern Denmark
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HCW	Health Care Workers
IB	Investigator's Brochure
IC	Informed Consent
IIV	Inactivated Influenza Vaccine
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
PI	Primary Investigator
PPV	Pneumococcus Polysaccharide Vaccine
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SPC	Summary of Product Characteristics
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization, or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor but referred to as a subsidizing party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
WHO	World Health Organization
WMO	Medical Research Involving Human Subjects Act

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SUMMARY

Background: The virus SARS-CoV-2 has spread rapidly throughout the world. Seniors are at high risk of severe COVID-19 when infected. Bacille Calmette-Guérin (BCG) is a vaccine against tuberculosis, with protective non-specific effects against other infections; significant reductions in morbidity and mortality have been reported, and a plausible immunological mechanism has been identified: “trained innate immunity”. We hypothesize that BCG vaccination can reduce the risk of COVID-19 and other infections among senior citizens during the COVID-19 pandemic.

Objectives: Primary objective: To reduce senior citizens’ risk of acute infection during the COVID-19 pandemic. Secondary objectives: To reduce senior citizens’ risk of SARS-CoV-2 infection during the COVID-19 pandemic. To reduce senior citizens’ risk of self-reported respiratory illness during the COVID-19 pandemic.

Study design: A placebo-controlled randomized trial.

Study population: 1900 seniors above 65 years of age.

Intervention: Participants will be randomized 1:1 to intradermal administration of a standard dose of BCG vaccine or placebo (saline).

Outcomes: Primary outcome: Acute infection identified either by a doctor, antibiotics use, hospitalization, or death due to infection. Secondary outcomes: Verified SARS-CoV-2 infection and self-reported respiratory illness.

With an expected incidence of acute infection of 20%, we will be able to show a 25% reduction in the risk of acute infection in the intervention group versus the placebo group by including a total of 1900 individuals, 950 individuals in each group.

Risk for participants and impact: Based on previous experience and randomized controlled trials in adult and elderly individuals, the risks of BCG vaccination are considered low. If BCG can reduce the acute morbidity in seniors by 25% it has tremendous public health importance, both during the COVID-19 pandemic and overall.

BACKGROUND

Worldwide, the population of persons>60 years has tripled since 1950, because of advances in average life expectancy. This transition is most advanced in high-income countries; already by 2030, seniors>60 years are expected to constitute >25% of the populations in Europe and US[1].

Immunosenescence

One of the most recognized consequences of aging is a decline in immune function, so-called "immunosenescence". Vaccination is the most effective prophylactic intervention for infectious diseases, but due to immunosenescence, the efficacy of vaccines decreases with increasing age[2]. Due to immunosenescence, severe infections are more common in the elderly. Not least during the COVID-19 pandemic, it has become clear that elderly people are particularly susceptible to severe COVID-19. Strategies to strengthen senior citizens' immune system are urgently warranted.

BCG vaccine

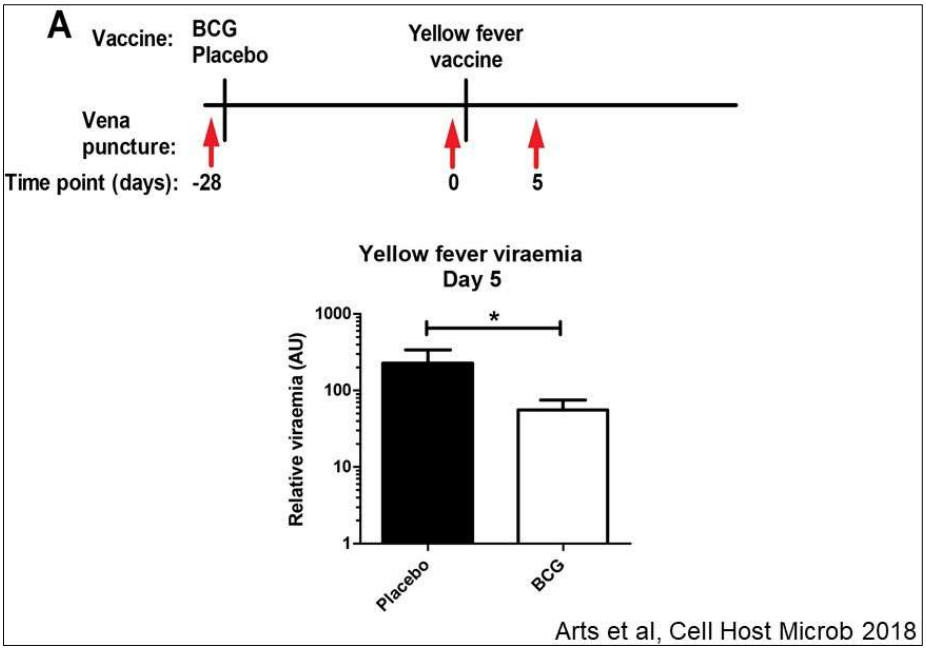
Bacillus Calmette-Guérin (BCG) was developed as a childhood vaccine against tuberculosis, but our group has shown that it can protect against death from other infections, i.e. it has what we have called non-specific effects (NSEs)[3]. In clinical studies, BCG vaccination was associated with decreased child mortality, mainly as a result of reduced neonatal sepsis and respiratory infections[4–6]. In a WHO-commissioned meta-analysis, BCG was associated with a 42% (95%CI: 24-55%) reduction in child mortality[7].

NSEs of BCG are not limited to children. An Indonesian trial with 34 subjects aged 60-75 years reported that compared with placebo, consecutive BCG vaccination for 3 months reduced the incidence of acute upper respiratory tract infections by 80% (95%CI=22-95%)[8]. Furthermore, a clinical trial performed in Japan found a reduced risk of pneumonia upon BCG vaccination in previously tuberculin-negative elderly people[9]. In a very recent clinical trial in Greece, BCG vs. placebo to senior citizens at discharge from the hospital was associated with a significant decrease in time to first infection (p: 0.035). The incidence of new infections was 42.3% (99% CIs 31.9 53.4%) in the placebo group and 25.0% (95% CIs (16.4-36.16%) in the BCG group; most of the protection was against respiratory tract infections (odds ratio 0.20; p: 0.001). No difference in the

frequency of adverse effects was found between groups. These data show that BCG vaccination is safe and can protect the elderly against infections (Mihai Netea, personal communication, paper in press).

In animal models, BCG has been shown to protect against influenza[10]. In a recent experiment, adults randomized to BCG vs. placebo 4 weeks prior to a challenge with a yellow fever vaccine had lower yellow fever viral load, and improved anti-viral responses (Figure 1)[11].

Figure 1: Healthy volunteers were injected with either placebo (n=15) or BCG (n=15). One month later all volunteers were injected with yellow fever vaccine. Viremia was assessed by PCR on day 5 after yellow fever vaccination. BCG vaccination significantly decreased viremia[11].



Immunological studies have now provided an explanation for the observed NSEs of BCG: NCG induces epigenetic and metabolic reprogramming of innate immune cells such as myeloid cells and NK cells, leading to an increased antimicrobial activity, a process termed 'trained immunity'[12]. Upon stimulation with a pathogen, the innate immune system becomes primed and is able to react faster and more efficiently to a secondary (and non-related) stimulus. These effects have

been shown to last for at least a year[12]. Presumably, the effect is maintained for so long because BCG vaccination induces a persistent transcriptional program connected to myeloid cell development and function within the hematopoietic stem and progenitor cell (HSPC) compartment in the bone marrow[13].

In a pilot study we recently investigated whether BCG could induce innate immune training in seniors above 50 years of age in Guinea-Bissau (preprint available at https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3611329). Two months after vaccination, BCG recipients had increased release of the pro-inflammatory innate cytokines IL-1β, IL-6 and TNF-α to non-specific stimuli. These effects were more pronounced among those with a positive QuantiFERON at baseline. Thus, BCG vaccination can induce a 'trained immunity' phenotype in older adults. These effects were boosted in previously Mycobacterium tuberculosis exposed individuals.

BCG vaccine and COVID-19

Currently, numerous clinical trials are investigating the effect of BCG as a prophylactic treatment for health care workers of all ages. No results of these trials are available yet, but ecological analyses have suggested that countries with a functioning BCG vaccination program have lower COVID-19 mortality[14]. Furthermore, in a very recent follow-up study of three cohorts of healthy volunteers who either received BCG in the last five years or not, BCG vaccination was safe and not associated with increased incidence of symptoms during the COVID-19 outbreak in the Netherlands. In fact, BCG vaccination was associated with a decrease in the incidence of sickness during the COVID-19 pandemic (AOR 0.58, $P < 0.05$) (own study, Cell Reports Medicine, in press).

Conclusion

Based on the capacity of BCG to 1) reduce the incidence of morbidity and mortality due to infectious diseases in children and in the elderly; 2) reduce viremia in an experimental human model of viral infection; 3) induce trained innate immunity in adults, also those previously exposed to mycobacteria; and 4) the fact that BCG given to elderly seems to be very safe, we hypothesize that BCG vaccination may strengthen the immune system of the senior citizens and may (partially) protect against getting infected and/or experiencing severe morbidity due to infections with SARS-

CoV-2 and other infectious pathogens. A randomized controlled trial provides the highest validity for this research question.

OBJECTIVES

Primary objective: To reduce senior citizens' risk of acute infection during the COVID-19 pandemic.
Secondary objectives: To reduce senior citizens' risk of SARS-CoV-2 infection during the COVID-19 pandemic. To reduce senior citizens' risk of self-reported respiratory illness during the COVID-19 pandemic.

HYPOTHESIS

BCG vaccination of seniors will reduce the risk of acute infection by 25% over a period of 12 months.

PROJECT GROUP

Christine Stabell Benn (MD, DMSc), Peter Aaby (DMSc), Anne Marie Rosendahl Madsen (MD, PhD student), Mette Bliddal (PhD), Sebastian Nielsen (MSc, statistician), and Frederik Schaltz-Buchholtzer (MD, PhD), all from University of Southern Denmark. Lene Annette Norberg (MD, PhD) and Anne Grete Pilgaard from Municipality of Odense. Mihai Netea (MD, DMSc), Radboud Medical Centre, Nijmegen, The Netherlands. Tyra Grove Krause (MD, PhD), Statens Serum Institut.

METHODS

Study design and follow-up

A randomized placebo-controlled clinical trial. We will enroll participants from September 2020 to August 2021.

Participants will be followed for 12 months post-randomization with respect to illness, medical contacts, use of antibiotics, hospitalization, and death. The follow-up will take place both through self-reporting, and through the Danish National Registers. Information on hospitalizations for infections and other medical conditions will be obtained through Denmark's National Patient Register[15] and information on use of antibiotics from the Danish Prescription Register[16]. Vaccination history will be acquired from the Danish Vaccination Registry at Statens Serum

Institut. Furthermore, data on testing for SARS-CoV-2 and results will be obtained via the local department of clinical microbiology.

Since the Investigational Medicinal Product (IMP), the BCG vaccine, is used in this study on another indication than the one it has been approved for, this is classified as a phase III study.

Study population

The trial will be implemented in cooperation with the Municipality of Odense and possibly also other municipalities, depending on the interest. Around 34,000 people ≥65 years of age live in Odense. The Municipality of Odense supports the operation of 14 activity houses, which are run by volunteers and host many different associations and activities. If we decide to include participants in other municipalities, we will seek similarly appropriate inclusion sites.

We plan to include 1900 senior citizens ≥65 years. Recruitment of study participants will take place at the senior activity houses and other places where senior citizens meet. In order to secure sufficient recruitment, we will apply for permission to contact potential participants directly via e-Boks. Potential participants will be sent an invitation to participate in the study based on a list of randomly selected CPR-numbers provided by the health authorities (Sundhedsdatastyrelsen). The letter of invitation will clearly state the purpose of the study and that participation is voluntary. Contact information is provided. If interested, the person will be given additional information, written and oral, about the study before they make their decision on whether to participate.

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet the following criteria: ≥65 years old.

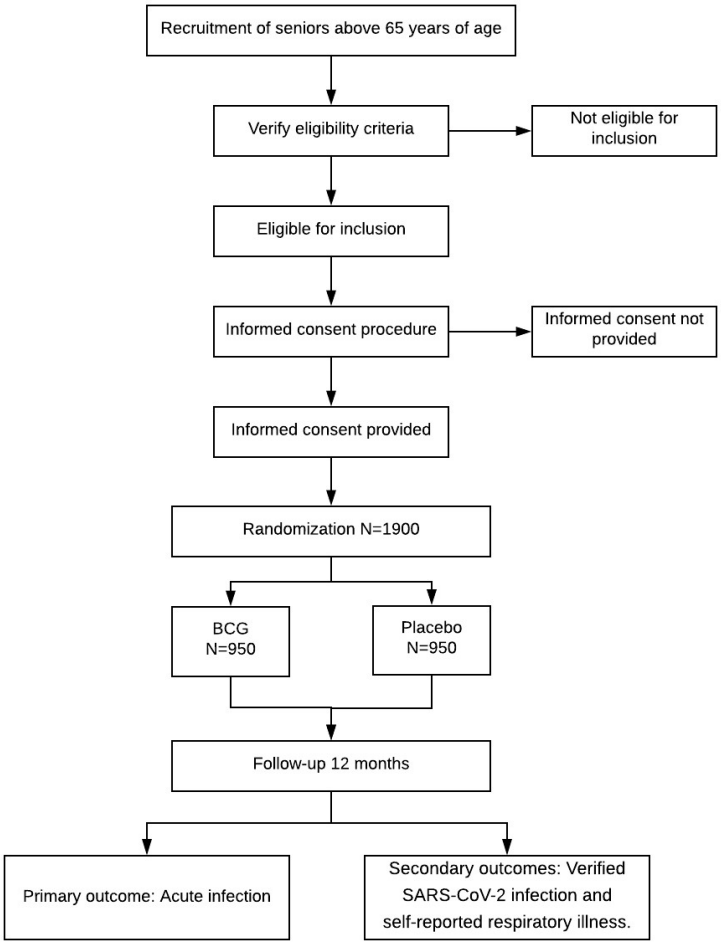
Exclusion criteria

The exclusion criteria will be assessed at the recruitment interview. We will not use the electronic patient records to check exclusion criteria. A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Known allergy to (components of) the BCG vaccine or serious adverse events in relation to prior BCG administration
- Previous *Mycobacterium tuberculosis (M. tuberculosis)* infection or known active or latent infection with *M. tuberculosis* or other mycobacterial species
- Fever (>38 C) within the past 24 hours or suspicion of active viral or bacterial infection
- Vaccination with other live attenuated vaccine within the last 4 weeks

- Severely immunocompromised subjects. This exclusion category comprises:
  - Subjects with known infection with the human immunodeficiency virus (HIV)
  - Subjects with solid organ transplantation or bone marrow transplantation
  - Subjects under chemotherapy
  - Subjects with primary immunodeficiency
  - Treatment with any anti-cytokine therapy within the last year
  - Treatment with oral or intravenous steroids defined as daily doses of 10 mg prednisone or equivalent for longer than 3 months
  - Active solid or non-solid malignancy or lymphoma within the prior two years
- Subjects who do not have access to e-Boks.

Figure 2: Recruitment and randomization.



Treatment of subjects

Participants will be randomized 1:1 to receiving an intradermal BCG vaccine or placebo. Participants who are randomized in the active arm will receive a BCG vaccine (BCG-Denmark, AJ Vaccines, http://www.produktresume.dk/AppBuilder/login.html). The BCG vaccines will be handled in full compliance with the requirements of the Summary of Product Characteristics (SPC). The vaccines provided by AJ Vaccines for this study (batch number 119009B and 118019D) has not been released for sale in Denmark, but has been released by WHO, certificate number: 2019093213 and 2019053438. The product is identical to the vaccine released for Denmark with respect to production, control and contents. AJ Vaccines will send the vaccines to Hospital Pharmacy of Funen. The pharmacy will assure correct control and storage of the vaccines and will release the vaccines to the study according to GCP. A collaboration agreement will be made with the pharmacy.

Placebo will be 0.1 ml sterile 0.9 % NaCl, which has a similar color as the resuspended BCG vaccine.

Description of route of administration and dosage

All participants will receive one injection at inclusion. No further treatment of study participants will take place. BCG will be administered in the upper arm, intradermally, 0.1 ml of the suspended vaccine. Placebo will be administered in the upper arm, intradermally, 0.1 ml of sterile 0.9 % NaCl solution. Immediately after injection, a pale papule usually occurs in the skin where the vaccine was given, as a sign of correct intradermal application.

STUDY PROCEDURES

See figure 3 below for flowchart of interventions.

Before participation

We will arrange meetings where information about the study is given to groups of interested citizens, provided this is possible with respect to infectious disease precautions. We will announce the study with posters in the local area and in relevant media: local newspapers, relevant internet media, as well as homepages and newsletters of the activity houses. It will be emphasized that follow-up to some extent will take place electronically via e-Boks; thus, it would be good if participants are familiar with this. Otherwise, help can be obtained at the houses. Citizens wishing

to participate will be given written information and will be booked for an interview. They will be informed about their right to bring a friend or family member. At the interview oral information will be given and eligibility will be assessed. The interview will take place by phone or in a private room and it will be possible to ask questions. The possible participants will then be offered reflection time of a minimum of 24 hours. If still interested they will be booked for inclusion.

Day of inclusion (day 0)

Study physicians, who are trained in good clinical practice and in providing intradermal vaccines, will be responsible for the inclusion of study participants. Enrolment will take place in a private room, and it will be possible to ask further questions. Informed consent will be obtained from all participants. Background information on participants will be collected in an electronical case report form system (REDCap). A blood sample of approximately 5 ml will be drawn for subsequent testing for SARS-CoV-2 antibodies.

Randomization and blinding

The study will be individually randomized, and placebo controlled. Randomization will be done using the REDCap tool with stratification per sex and age groups (65-74/75+ years of age) in randomly selected block sizes of 4 and 6.

The participant will be blinded to the treatment. They will be asked to leave the room while the vaccine/placebo is prepared. Once ready for injection, the vaccine and placebo will look similar, and the participant will not be able to tell the difference. The physicians administering the BCG vaccine or placebo will not be blinded. In case of serious adverse events, the participant can be unblinded after consultation with the PI or sponsor.

Day of inclusion till end of trial

A short electronic questionnaire regarding health, symptoms and potential side effects will be sent to the participants biweekly. Link to the questionnaire will be sent via e-Boks.

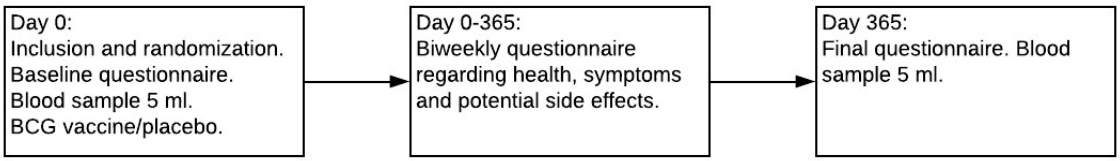
End of trial

Participants are asked to fill in a final questionnaire. A blood sample of approximately 5 ml will be drawn for subsequent testing for SARS-CoV-2 antibodies.

The end of the trial is defined as whichever comes latest: The last participants last registration in the online data collection, or 365 days. When the study is ended, all participants receive an email

with information about the intervention that they have received (BCG/placebo).

Figure 3: Interventions.



Loss to follow-up

In case of not filling in the questionnaire for 7 days, the participant receives a reminder in their e-Boks with a new link to the questionnaire. Participants with incomplete follow-up despite reminders will be telephoned with the request to complete the follow-up data. Permission for this procedure will be requested from the participant at enrolment.
Subjects can leave the study at any time for any reason if they wish to do so, without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. A participant will only be replaced in case of withdrawal before randomization. The participant will be asked to give a blood sample for SARS-CoV-2 antibody testing at end of the study period – even if they for some reason leave the study prematurely.

LABORATORY ANALYSES

The blood samples taken at inclusion and by end of trial will be analyzed for SARS-CoV-2 antibodies to assess the level of SARS-CoV-2 infection and/or vaccination in the participants.
We will also investigate the effect of BCG vaccination on the immune system by assessing the effect on DNA-methylation (one of the regulatory epigenetic mechanisms of gene transcription, including modulation of immune responses) and on inflammation biomarkers in the plasma before and after vaccination.
DNA will be extracted for assessment of DNA methylation profiles. DNA methylation will be measured using the Illumina Infinium Human MethylationEpic BeadChip. The samples will be randomized on the array to avoid batch effects. DNA methylation is a biomarker of gene transcription that mirrors exposure to various non-genetic factors, rather than genetic make-up of

individuals.

For age prediction, methylation age will be determined using the measurement from the pre-defined marker CpG sites. Specifically, the online calculator (https://dnamage.genetics.ucla.edu) or the ageP function from the R Watermelon package will be used. We will focus on the data from those marker CpG sites (between 300 -1000 epigenetic markers) and use them to predict DNA-methylation age of those samples, rather than a widespread genome mapping analysis.

The importance of assessing DNA-methylation and inflammatory biomarkers is to understand the mechanisms that mediate the effects of BCG vaccination. It does not have any known impact on disease pathophysiology. The assessment of immunological parameters thus has no consequences for the health status of the volunteers and are exclusively part of the scientific discovery component of the project.

OUTCOMES

The primary outcome is acute infection identified either by a doctor, antibiotics use, hospitalization or death due to infection. An acute infection event identified by a doctor is defined as newly onset illness that has prompted the participant to seek medical attention from general practitioner or on-call doctor. Repeated symptoms will be defined as a new event when separated from original illness by 7 days or more. Information on visits to general practitioners are not available from national registers, hence this parameter is based on self-reporting. Information on acute infection identified by a doctor will come from the questionnaires, where participants are asked about symptoms and whether they have needed to see a doctor. Information on antibiotics use, hospitalization and death will be acquired from the Danish National Registers.

Secondary outcomes are on the clinical level verified SARS-CoV-2 infection and self-reported respiratory illness, while on the mechanistic level it will be the DNA-methylation of immune cells and inflammatory biomarkers in plasma before and after vaccination. Supplementary analyses will explore the effect of the intervention on severity of any SARS-CoV-2 infection.

Self-reported respiratory illness is based on information on symptoms given by the participants in the biweekly questionnaire.

Senior citizens are offered the seasonal inactivated influenza vaccine (IIV) and as of last year they

are also offered the pneumococcal polysaccharide vaccine (PPV). Furthermore, the specific COVID-19 vaccines are now available to the participants. This means that some participants will be enrolled prior to these vaccinations and some after. We will register these and other vaccinations the participants might receive during the study period but will not interfere with the standard vaccinations. This allows for an analysis of potential interactions and the possible effect the sequence of vaccinations may have on the outcome.

STATISTICAL ANALYSIS

The primary endpoint “acute infection” will be analyzed as a recurrent time-to-event using an Andersen-Gill Cox proportional hazards regression model with time since inclusion as underlying time scale. Analyses will be done stratified by the block randomization variables sex and age group.
The analysis will be done with and without censoring for subsequent vaccines, and also with and without stratification by most recent prior vaccine, to assess possible interaction between BCG and other vaccines.

The secondary endpoint self-reported respiratory illness will be analyzed the same way as the primary endpoint. The other secondary outcome verified SARS-CoV-2 infection will be analyzed in a standard Cox proportional hazards model, but otherwise as described above.
All data will be analyzed stratified by previous BCG vaccination (self-reported/assessed by BCG scar) to assess the effect or revaccination vs. first vaccination with BCG.

When applicable (i.e., in the event that one or more participants have died during the follow-up period) a competing events analysis will be performed in addition (Fine-Gray model).

Other study parameters: continuous baseline characteristics will be reported as mean and standard deviation or median and inter-quartile range, as appropriate. Categorical baseline characteristics will be reported as count and percentage. No statistical testing for baseline characteristics will be performed.

Data will be reported quantitatively. All analyses will be performed from the intention-to-treat principle. Missing data will be dealt with by multiple imputation using the mice package in R.

SAMPLE SIZE CALCULATION

With an expected incidence of “acute infection” of 20% we will be able to show a 25% reduction in the risk of acute infection in the intervention group versus the placebo group by including a total of 1890 individuals, 945 individuals in each group. Due to the fact that the primary outcome data are to a large extent obtained via national registers, we anticipate limited loss of power due to loss to follow-up. We therefore aim to enroll 1900 participants.

SAFETY REPORTING

Temporary halt for reasons of subject safety

In accordance with section 10, subsection 4, of the Medical Research Involving Human Subjects Act (WMO), the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the EC and the DKMA without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the EC and the DKMA. The investigator will take care that all subjects are kept informed.

Adverse events (AEs): Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product, the placebo or the trial procedures. We will only report AEs to the Ethics Committee (EC) in the end of trial report.

Serious adverse events (SAEs): A serious adverse event is any untoward medical occurrence or effect that:

- Results in death
- Is life threatening (at the time of the event)
- Requires hospitalization or prolongation of existing inpatients’ hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Or any other important medical event due to the intervention based upon appropriate judgement by the investigator

An elective hospital admission will not be considered as a serious adverse event. Participants will

be asked about the occurrence of SAE's biweekly in the questionnaire. In case of a SAE, dependent on the symptoms of the participant, he/she will be contacted by the investigator. The condition of the participant will be evaluated by the investigator, who can decide to un-blind if deemed necessary. In case a participant stops filling in the online questionnaires, he or she will be called by the investigator to collect data on potential SAE's.

The investigator will notify sponsor within 24 hours after first knowledge of any SAEs, in order for sponsor to assess whether it is a SUSAR.

Serious adverse reactions (SARs) and suspected unexpected serious adverse reactions (SUSARs):

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered. The BCG vaccine SPC will be used as reference. Unexpected adverse reactions are SUSARs if the following three conditions are met:

- The event must be serious (see above)
- There must be a certain degree of probability that the event is a harmful and undesirable reaction to the medicinal product under investigation, regardless of the administered dose
- The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction is not in agreement with the product information as recorded in the SPC

The investigator and the sponsor will report SUSARs to the EC and the Danish Medicines Agency (DKMA). The sponsor will ensure that all relevant information about SUSARs that are fatal or life-threatening is recorded and reported as soon as possible to the Danish Medicines Agency, and to the Ethics Committee, and in any case no later than seven days after knowledge by the sponsor of such a case. All other SUSARs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. Reporting will be done using the *eBlanket* provided by the DKMA. The investigator and the sponsor will report the following SUSARs to the EC and the DKMA:

- SUSARs that have arisen in this clinical trial assessed by the EC and DKMA
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in this clinical trial.

SARs and SUSARs are recorded in an overview list (line-listing) that will be submitted to the EC at the end of trial. This line-listing provides an overview of all SUSARs from the study medicine,

accompanied by a brief report highlighting the main points of concern.

It will not be possible to screen all questionnaires for possible SAEs/SARs in real time. We will therefore ensure timely follow up of these events by installing notification logics in REDCap. The primary investigator will get a notification by e-mail in case a subject reports suspicion of a severe side effect or has sought medical attention because of possible side effects or acute illness. Also, subjects are advised to contact the investigator in case of questions or doubts concerning the treatment or possible reactions following the treatment.

SAEs, SARs and SUSARs will be monitored for three months after inclusion. All SAEs, SARs and SUSARs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

ETHICAL CONSIDERATIONS

The study will be conducted according to the principles of the Declaration of Helsinki amended at the General Assembly in October 2013 and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. The study will be submitted for approval to the EC and DKMA. We will ask participants permission to access electronic patient records, in order to access relevant test results and information on hospital admissions in case of serious adverse events/reactions in the study period. We only need to access the records **after** the participants have given their consent. The participants will be informed, that their consent gives the sponsor, sponsors representatives, PI, and monitoring agencies direct access to relevant health information from their electronic patient records.

Benefits and risks assessment

The potential benefit for subjects randomized to the BCG-arm is possibly a lower risk of getting infectious diseases including potentially a lower risk of COVID-19. Potential risks include only the well-known side effects of the vaccine. The BCG vaccine SPC will be used as reference document. As part of the study information, participants will be informed about the possible side effects to the treatment, including that it is common and not treatment demanding that, following BCG vaccination, redness, swelling and ulceration of the skin where the vaccine is given will occur, that there may be swollen lymph nodes in the area some weeks after the vaccine is given, and that in

the longer term, a small scar, approximately 5 mm diameter, will appear where the vaccine was given. This kind of side effects will not be reported to DKMA.
Severe but rare side effects are injection site abscesses, BCG lymphadenitis, disseminated BCG disease, osteitis, osteomyelitis, anaphylaxis, formation of keloid/lupoid and suppurative lymphadenitis. A complete list of known side effects can be found in the participant information leaflet. Subjects that receive the placebo hardly have any potential risks and no benefits. Local hematoma formation can occur at the site of injection.
BCG vaccine in immunocompetent adult people is considered safe, also for persons with prior BCG vaccination and even in latently infected adults[17,18]. In a randomized controlled trial that compared revaccination with BCG versus placebo, no vaccine-related serious adverse events were observed in the 312 patients in the BCG arm[19].
Thus, the risk to and burden for the subject of BCG vaccination is estimated to be low, according to previous trials that have been performed with BCG vaccines[8,11,19]. It is not recommended to get other live attenuated vaccines within a month of BCG vaccination, unless given at the same time. Apart from this, no interactions are known between BCG vaccination and other medications.
Participation in this study should in principle not be an exclusion criterion for participation in other studies (for example into treatments for COVID-19). The Mantoux test for screening for tuberculosis is no longer reliable after BCG vaccination. The reliability of the TB-Quantiferon test is not affected by BCG-vaccination.

Compensation for injury

The study participants are fully insured according to the Danish Patient Insurance Act. This insurance provides cover for damage to research subjects through injury or death caused by the study. Participants will not be compensated for participating in this study. Damage to subjects through injury or death, caused by the study or negligence of local study investigators, is not accountable to the investigators.

ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

Questionnaire data and biological material will be registered and stored via OPEN (Open Patient data Explorative Network) at Odense University Hospital using REDCap, OPEN Analyze and OPEN

Biobank hosted by OPEN on secure servers in the Region of Southern Denmark. Questionnaire data and results from blood samples will be merged to register data from National Health registers at secure servers at the Danish National Health Authority and all data analysis using data from National registers will be performed at these servers. Data analysis will be done according to safety regulations with pseudonymized copies of data. Data will be processed and stored locked / inaccessible and safely in full compliance with the Data Inspectorate's Standard Terms for Research Projects. All source data will be kept in the electronic CRF in REDCap and the electronic Trial Master File (TMF) will be kept in a secure site in Sharepoint. The sponsor, investigators appointed by the sponsor, the study statistician, and monitoring agencies will be able to get direct access to source data.
The handling of personal data complies with the EU General Data Protection Regulation and the Danish Act on Implementation of the General Data Protection Regulation.
The TMF and the electronic data from the eCRF will be stored for a duration of 5 years.
Information, data, and results that originate from this study may not be disclosed without the written permission of the sponsor and coordinating principal investigator. The blood samples will be kept in an OPEN research biobank during the study period and potentially remaining biological material will be stored in an OPEN biobank for 5 years after end of trial, allowing for the possibility that new insights may be reached during this time, which could be addressed in the existing material. If that is the case, ethical permission to analyze the samples will be applied for. After 5 years all remaining biological material will be destroyed.

Monitoring and Quality Assurance

We classify this trial as a low-risk study, because the intervention is already registered, and has been used in previous trials. The study will be monitored by the GCP unit at Odense University Hospital. The first monitor visit will take place before inclusion of the first study participant. During this visit the presence and completeness of the relevant Study Files will be checked.

End of trial reporting

The sponsor will notify the EC and DKMA about the end of the study within a period of 90 days. Within one year after the end of trial, the sponsor will submit a final study report with the results

of the study, including any publications/abstracts of the study, to the EC.

The sponsor will notify the EC and DKMA immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the EC and DKMA within 15 days, including the reasons for the premature termination.

Public disclosure and publication policy: The results of this study will be disclosed unreservedly after the end of trial. Results that are important for public health will be notified to the competent authorities as soon as possible. The trial will be registered in a public trial registry before the first patient is recruited.

FUNDING AND MATERIAL SUPPORT

The study has been supported by “Independent Research Fund Denmark” (Danmarks Frie Forskningsfond). The funding will cover the running costs, vaccines, materials, analyses, statistical and data management support, and salaries. The vaccines have been bought from AJ Vaccines. This company has had no influence on the design of the study and will not have influence on any aspect of analyzing, interpretation or reporting of results. None of the parties in the study group have any economic interest in the company. The EC will be informed about any further economic or other support that the project may receive. This will include the amount received, how it will be used and account number.

MAJOR MILESTONES

Application for Regional ethical committee submitted: August 2020
Application for EudraCT submitted: August 2020
Application for Danish Medicines Agency submitted: August 2020
Inclusion of first participant: September 2020
Inclusion of last participant: August 2021
End of follow-up: September 2022
Data analyzed: February 2023

PERSPECTIVES

In this trial, the BCG vaccine will be used in an attempt to protect senior citizens from infections, especially from COVID-19. The risk associated with getting the BCG vaccine is low and only minor local side effects are common (redness, swelling, local pain). The benefits of BCG vaccination, based on earlier clinical studies, are expected to be a reduction in morbidity of 25% compared to unvaccinated individuals, and potentially higher. These effects are mediated by the capacity of BCG to induce trained immunity and may represent an important tool for protection against COVID-19 until a specific effective vaccine is developed and as a way to combat immunosenescence in general.

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