Using BCG vaccine to strengthen the immune system in the elderly and improve the response to influenza vaccine. A randomized clinical trial.

(BCG-DENMARK-INFLUENZA)

Danish title: "Immunforsvar mod influenza"

Hovedansvarlig og sponsor, initiativtager til forsøget:

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Jeg bekræfter hermed med min underskrift, at denne version af studie-protokollen er den gældende, og at vi vil udføre studiet i henhold hertil, i henhold til ICH-GCP guideline og gældende myndighedskrav/lovgivning.

7. oktober 2021: 

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7. oktober 2021: 

Forsøgssted:

OPEN, Klinisk Institut, Syddansk Universitet, står for at udføre forsøget, med satellit-rekrutteringssteder i Odense Kommunes frivillighuse.

Content

Abbreviations

AE	Adverse Event
AR	Adverse Reaction
BCG	Bacille Calmette Guérin
CRF	Case Report Forms
DSMB	Data Safety and Monitoring Board
IIV	Inactivated Influenza Vaccine
PBMCs	Peripheral Blood Mononuclear Cells
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reactions

OBJECTIVES

We will test the effects of BCG vaccine on the specific immune response to seasonal influenza vaccination and other subsequent inactivated vaccines, as well as on the immune system and health in general in elderly people ≥ 65 years, with the aim to

a) improve their specific antibody response to vaccination

b) improve their general resistance towards infections

c) study the effect of BCG on classical lymphocyte-dependent responses, and the induction of innate immune memory.

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, elderly > 60 years are expected to constitute > 25 % of the populations in Europe and US[1].

Immunosenescence

One of the most recognised 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.

Response to influenza vaccine in the elderly

Due to immunosenescence, severe influenza virus infections are more common in the elderly. It is therefore generally recommended that everyone > 65 years receives the seasonal inactivated influenza vaccine (IIV)[2]. However, in a review of 31 studies, the adjusted odds-ratio for seroconversion and seroprotection to influenza vaccine antigens in elderly versus young adults ranged from 0.24 to 0.59[3]. Correspondingly, although influenza vaccination of young adults provides 65–80 % protection against illness, vaccinating elderly only affords 30–50 % protection[4].

Combatting immunosenescence

It is important to identify novel approaches to strengthen vaccine responses and to improve the general resistance in the elderly. In this respect, the new concept of "trained immunity" offers an attractive target for immunomodulation: epigenetic and functional reprogramming of innate

immune cells has been shown to induce broad heterologous protection against infections[5].

In the present project, we will explore Bacille Calmette-Guérin (BCG) as a low-cost, low-risk intervention, based on "trained immunity", to increase the immune response to vaccines and improve the general resistance towards infections in elderly.

Proposed intervention: BCG vaccine

The BCG vaccine (the "*Calmette* vaccine") was developed for tuberculosis prevention. However, there is increasing evidence that it also has beneficial heterologous "non-specific" effects on the immune system[6,7]. Studies among children in low-income countries have shown that the BCG vaccine reduces neonatal mortality by 38 % (95 % CI: 17-54 %), far more than can be explained by prevention of tuberculosis; BCG protected against septicaemia and respiratory infections[8].

It has been suggested that this effect of BCG is at least partly due to the induction of trained immunity[9,10]. Laboratory studies have shown that BCG vaccine strengthens the innate immune system with increased activity against non-related infections[9,11]. The trained innate immune cells are more potent in activating specific lymphocyte responses. A potentiating effect of BCG vaccine on the response to vaccines has been seen in studies in infants, where BCG vaccination increased heterologous antibody responses to vaccination with polio vaccine[12] as well as pneumococcus, *Haemophilus influenzae* type B and tetanus toxoid vaccines[13], and Hepatitis B vaccine[14].

In a recent experimental study, BCG vaccination influenced antibody responses to IIV[15]. Dutch volunteers were randomised to either BCG (n=20) or placebo (n=20), followed by IIV 2 weeks later. In BCG-vaccinated subjects, antibody responses against IIV were significantly enhanced, and seroconversion was borderline significantly increased (85 % of BCG-vaccinated subjects seroconverted versus 65 % in the placebo group (p=0.08))[15].

The non-specific effects of BCG have mostly been studied among children. To our knowledge only two studies explored the non-specific effect of BCG vaccination on heterologous infectious disease morbidity in the elderly. In an Indonesian study of 34 elderly, of whom half were BCG vaccinated monthly for 3 months, BCG was associated with a significant reduction in the risk of acute upper respiratory tract infection from 59 % to 12 %[16]. A recent Greek study included 202 elderly patients randomized to BCG vaccination or placebo at discharge from hospital. The risk of acquiring a new infection within a year was reduced by 45% in the BCG group and the risk of respiratory infection reduced by 79%. Also the time until next hospital admission was significantly increased in the BCG

group (16 versus 11 weeks)[17].

Known side effects and risks associated with BCG

The BCG vaccine has been used for almost 100 years and is now part of more than 100 countries' childhood immunization programs. In Denmark, we gave it up to the 1980s, after which we stopped, as the tuberculosis risk decreased. The following side effects are common: Redness and swelling of the skin where the vaccine is given, swollen lymph nodes up to 4 weeks after the vaccine, and a small ~5 mm scar.

Because the vaccine has been known and used for many years, we also know the rare side effects: Less than 1 out of 100 can get headaches, fever or wounds above the vaccination site. Less than 1 in 1,000 can get a boil over the vaccination site, and less than 1 in 25,000 can get bone inflammation, lymph node inflammation or severe allergic reaction.

Conclusion

There is an urgent need to counteract the consequences of the increasing population of elderly persons, who are at higher risk of infectious disease morbidity and mortality. BCG vaccine represents a promising intervention, which in small studies improved the immune response to IIV and reduced the risk of infections in general. The intervention is low- cost, very acceptable and safe, also in the weakest individuals, such as infants and elderly.

HYPOTHESES

We will test the following hypotheses:

a) BCG vaccine given 2 weeks prior to, together with, or 2 weeks after IIV is associated with a 30% increase in seroconversion to IIV and/or a significant increase in influenza antibody titres.

b) The hypothesised potentiating effects of BCG is due to a combination of classical lymphocyte-dependent responses, and the induction of innate immune memory (trained immunity) in myeloid cells.

PROJECT GROUP

Sponsor: Christine Stabell Benn, University of Southern Denmark. Prof. Benn has decades' experience conducting large-scale randomised trials to study non-specific effects of vaccines.

Primary investigator (PI): Anne Marie Rosendahl Madsen, MD, PhD-student, University of Southern Denmark. The project will be a part of her PhD project.

Mihai Netea, Radboud University. Prof. Netea described the epigenetic mechanisms mediating innate immune memory ("trained immunity") for the first time and has mapped BCG vaccine's effects on the innate immune system. Prof. Netea will be responsible for the sub study of innate immune training.

Torben Barington, Clinical Immunology, University of Southern Denmark/Odense University Hospital. Prof. Barington will be responsible for processing and analysis of biological material and the sub study of activated lymphocytes.

Lene Annette Norberg, Municipality of Odense. The project would not be feasible without the assistance from the Municipality of Odense.

Other collaborators:

Odense Patient data Explorative Network (OPEN), Odense University Hospital, Region of Southern Denmark, will assist with data management as well as provide storage of data and biological material. ***Statens Serum Institut, Copenhagen*****,** will supply influenza vaccines for the study. ***The pharmacy at Odense University Hospital, "Sygehusapotek Fyn",*** will help with storage of vaccines and study medication and preparation of labels for study medication.

The trial will be monitored by the ***GCP unit at Odense University Hospital***.

METHODS AND STUDY DESIGN

The proposed project is a single-blinded randomized clinical trial, with nested immunological studies.

Participants will be randomized 1:1:1:1 to four groups: BCG 14 days before, at the same time, or 14 days after IIV compared to placebo. In the event that COVID booster vaccines are recommended we will aim to also explore the effect of BCG on the immune response to these vaccines. We aim to include 75 participants in the three groups, 300 in total.

Figure 1. Basic study design:

Group	Day 0	Day 14	Day 42
1	BCG	IIV+Placebo	Follow up visit
2	Placebo	IIV+Placebo	Follow up visit
3	Placebo	IIV+BCG	Follow up visit
4	IIV	Placebo+BCG	Follow up visit

The Primary Investigator (PI) and a statistician will prepare the recruitment, enrolment and follow-up of participants in the randomised trial. This includes developing the case report forms (CRF) and the REDCap tool to be used for randomisation, data capturing at study visits, and self-reporting of illness by participants.

Setting and recruitment

The randomised clinical trial will be implemented in cooperation with the Municipality of Odense. Around 35,000 elderly > 65 years 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. These houses are very popular among the elderly. Recruitment, enrolment, and follow-up with collection of biological material will be conducted at some of these houses.
The project will be presented at special arrangements in the houses, if possible, with respect to potential restrictions related to the COVID-19 epidemic. Information about the study will be delivered as outlined in the written participant information.
Potentially interested participants will be given the written participant information and an empty consent form. They will be provided with a telephone number for further contact and information. If still interested in participating after 24 hours, they will be given the possibility of signing up for study day 0 (see below) either online or by telephone.

Eligibility and exclusion criteria

Inclusion criteria:

Elderly people > 65 years and eligible for seasonal IIV. Participants must have access to ‘e-Boks’.

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.
- Participant in BCG-DENMARK-SENIOR study

Informed consent

As described previously, oral information about the study will be provided during the presentation of the project at special arrangements in the activity houses. It will be emphasised that follow-up to some extent will take place electronically; thus, it would be good if participants are familiar with this; otherwise, help can be obtained at the activity houses. Interested citizens will receive written information and consent form. Citizens wishing to participate will be asked to book a consultation on one of the recruitment days (day 0), and they are informed about their right to bring an assessor. They are offered at least 24 hours to consider the oral and written information given, before signing the consent form. They will be asked to bring the signed consent form with them on the recruitment day.

Time plan

For logistical reasons, we will strive to enrol participants over two-four weeks, still respecting the

recommended time of seasonal IIV in October/November 2021 (Figure 2).

Day 0

The recruitment takes place in the activity houses. The PI and staff trained in recruitment, good clinical practice and in providing intra-dermal vaccines, will be responsible for the recruitment. The recruitment will take place in a separate room. The potential participants will have an individual consultation, where it will be possible to ask further questions, and the PI can assure eligibility.

Collection of background data

At inclusion, background information on participants, including information on prior influenza vaccination with vaccine strains, will be collected. The study data will be collected in an electronical case report form system (REDCap).

Figure 2. Flow of participants per week from recruitment at day 0 until day 42.

Week number	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
First date in week	27/9	4/10	11/10	18/10	25/10	1/11	8/11	15/11	22/11	29/11
Info meetings	x	x								
Randomization (day 0)			150	150						
Influenza vaccination (day 14)					150	150				
Follow-up visit (day 42)									150	150
Antibody blood draws day 14					150	150				
Antibody blood draws day 42									150	150
Blood draws day 21 (n=10 from group 1-3 = 30)						15	15			
Blood draws day 0 (n=15 from each group = 60)			30	30						
Blood draws day 14 (n=15 from each group = 60)					30	30				
Blood draws day 42 (n=15 from each group = 60)									30	30
Lymfocyte assay blood draws										
Innate immune training blood draws										

Randomisation

The study will be individually randomised, and placebo controlled. Randomisation will take place using REDCap. The randomisation will be done in blocks of 6-8, stratified by previous influenza vaccine, sex and age group (65-74; 75+). As described above, participants will be randomised to

one of the 4 groups (Figure 1) and given the allocated treatment. A subgroup of 15 in each group will have a blood draw of 20 ml (Figure 2).

Blinding

It is not possible to blind the MD providing the treatment, but study participants will be blinded with respect to treatment allocation.

Interventions and doses

The BCG vaccine will be the danish BCG vaccine (BCG Denmark, AJ Vaccines, batch number 120016D and 120016B) provided by intradermal injection on the upper arm in a dose of 0.1 ml as recommended for adults; placebo will be the same volume of saline.

The product summary for the BCG vaccine is enclosed. The vaccines will be labelled by the pharmacy at Odense University Hospital. The vaccines provided by AJ Vaccines for this study has not been released for sale in Denmark, but has been released by EU, certificate number: 2020071305. The product is identical to the vaccine released for Denmark with respect to production, control, and contents.

The BCG vaccines will be handled in full compliance with the requirements of the SPC, including:

- Do not mix with other medicines.
- Store for a maximum of 18 months at 2-8 degrees in original packaging protected from light.
- Used max 4-6 hours after re-constitution.

Immediately after an intradermal injection with 0.1 ml, a pale papule usually occurs in the skin where the vaccine was given. To monitor the quality of the intradermal injection, we will measure and record the size of the papule and take a photo of it. The photos will be close-up photos of the papule and they will thus not be personally identifiable.

Post-vaccination, participants will be asked to wait 15 minutes in a waiting room outside the recruitment room before leaving.

Day 14

Participants will be asked to come back for the seasonal IIV on day 14. A blood sample (20 ml) will be obtained from all participants. Furthermore, all participants will receive an intra-dermal injection with BCG (group 3 and 4) or saline (group 1 and 2) in the opposite arm. Again, participants will be blinded to the treatment. The vaccination papule will be photographed/registered. Information on potential symptoms during the preceding 2 weeks will

be collected by means of questionnaires (REDCap).

Following this, the IIV will be administered intramuscularly in the recommended dose. Group 4 will have a placebo injection as they have received the IIV at day 0.

The influenza vaccine will be the vaccine recommended for the coming influenza season by the Danish health authorities (Influvactetra), in the recommended dose. It will be provided, via Statens Serum Institut and handled according to the recommendations. The vaccines will be labelled by the pharmacy at Odense University Hospital. The product summary for Influvactetra is enclosed.

The influenza vaccine will be handled in full compliance with the requirements of the SPC.

Post-vaccination, participants will be asked to wait 15 minutes post-vaccination in a waiting room outside the recruitment room before leaving.

Day 21

A subgroup of 10 individuals from each group (See Figure 2) will be asked to come back for another blood sample (20 ml).

Day 42

All participants will be asked to come back for another blood sample (20 ml). Information on potential symptoms during the preceding month will be collected by means of questionnaires (REDCap).

Follow-up to 6 months post-randomisation

Further follow-up of all participants to 6 months post-randomisation will take place through self-reporting. Questionnaires will be sent to the participants biweekly via e-Boks. After the 6 months, a final questionnaire will be sent to the participants.

Access to Electronic Patient Records

We will ask for permission to access electronic patient records, in order to assess how many of the participants have been tested for influenza and/or have been admitted to hospital with influenza like illness or other diseases within the follow-up period. The PI will gain access to electronic patient records via OPEN Odense University Hospital. Testing for influenza on Funen, both in general practice and at hospitals, is mainly done via the department of clinical microbiology at Odense University hospital, so test results will be available in the laboratory data system. We will ask for permission to gain access to test results via the laboratory data system. A collaboration agreement will be made with the department.

Information on previous influenza vaccination and vaccine strains as well as other vaccinations will be obtained from the Danish Vaccination Registry, in which all vaccinations have been registered since November 2015.

Potential loss-to-follow-up

Participants are informed that they can withdraw from the study at any given time, without any consequences. Their data will be used up to the time point where they withdraw, unless they do not wish that to be the case. We will not compensate for the potential loss of participants by including new participants.

Blood samples

Pseudonymised blood samples will be analysed at the Department of Clinical Immunology at Odense University Hospital and at Radboud University in The Netherlands. Collaboration agreements and data processor agreements will be made between OPEN and the two laboratories. The samples that are shipped to The Netherlands will be handled according to the laws and regulations in The Netherlands, which are similar to those in Denmark. 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 analyse the samples will be applied for. After 5 years, all remaining material will be destroyed.

Laboratory analyses

Antibody titres towards the influenza vaccine antigens will be measured in hemagglutination-inhibiting assays according to standard procedures. Geometric mean titres will be determined by calculating the mean of the log-transformed duplicate titres followed by back transformation. Seroconversion will be defined as ≥4-fold titre increase, compared with baseline[15].

In a subgroup of 15 participants from each group (the first 15 to accept), the immune function will be assessed by cytokine production capacity measured by Luminex technology after stimulation with non -related bacterial, viral and fungal stimuli. Venous blood will be drawn into EDTA tubes, peripheral blood mononuclear cells (PBMCs) will be isolated and stimulated with various antigens and mitogens as done in previous studies[9,15]. We will also stimulate PBMCs with influenza vaccine (the same batch of IIV administered to participants). In addition, we will measure inflammatory mediators in the blood, as well as transcriptional and epigenetic profiles of the myeloid cells ("trained innate immunity") as done in previous studies[9]. In the subgroup of 10 individuals from

each group that were bled at day 7 post-influenza vaccination, we will examine activated, influenza antibody-secreting B lymphocytes for isotype (IgM, IgG, IgA) using ELISPOT technique, and VDJ-repertoire, CDR3 length distribution and presence of somatic hypermutations by next-generation-sequencing VDJ DNA and mRNA from influenza-specific B blasts purified from blood by influenza antigen-coated immunomagnetic beads.

OUTCOMES

The primary outcome will be change in antibody levels to influenza virus strains, comparing levels just before and 4 weeks after IIV.

Secondary outcomes will be:

- Infection rate for 5 months post-randomisation
- Association between influenza antibody level and subsequent infection rate
- Lymphocyte-dependent responses 7 days after influenza vaccination
- The induction of innate immune memory 14- and 42-days post-randomisation

Infection rate is based on information given by the participants in the follow up questionnaires. This includes self-reported symptoms, visits to general practitioner or on call doctor, as well as information on hospitalisations. Repeated symptoms will be defined as a new event when separated from original illness by 7 days or more. In case of hospitalisation, further information on diagnoses and relevant test results will be obtained from the electronic patient records. Information on test results concerning influenza and other respiratory pathogens will be obtained for all participants.

STATISTICAL METHODS AND SAMPLE SIZE

Antibody levels will be compared between groups in regression analyses with adjustment for baseline levels. The proportion of participants who seroconvert will be compared in logistical regression analysis. Based on previous studies, which had 15 individuals in each group, [15], we should be able show an effect of BCG on the immune response to influenza vaccine with 75 individuals in each group. With an anticipated seroconversion of 50 % in the control group, we should be able to show a 30 % increased seroconversion associated with BCG vaccine with 80 % power, an alpha of 0.05 and anticipating 10 % loss to follow-up.

The immunological studies will provide information on the immunological effect of the

interventions, including their ability to induce trained innate immunity.
Lastly, all participants will be followed for clinical symptoms for 5 months. With 75 individuals in each group, we can only show differences in clinical symptoms if such differences are pronounced but given that a previous study showed effects with less than 50 individuals in each group[16], it seems worthwhile. A double-sided p-value of < 0.05 will be considered statistically significant.

DATA MANAGEMENT AND STORAGE

Data and biological material will be registered and stored via OPEN, Open Patient data Explorative Network, using REDCap, OPEN Analyse and OPEN Biobank hosted by OPEN on secure servers in the Region of Southern Denmark. Data analysis will be done according to safety regulations with pseudonymised 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, including PhD student and a 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.

MONITORING AND STOPPING RULES

The trial will be monitored by the GCP unit at Odense University Hospital. Due to the short duration of the study (6 months), interim analyses are not planned, and annual safety report will not be submitted. 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.

ETHICAL CONSIDERATIONS

The study will be submitted for evaluation by the Ethical Committee and by the Danish Medicines Agency. We will ask participants permission to access electronic patient records, as described above. 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 and monitoring agencies direct access to relevant health information from their electronic patient records.

Risks for participants

The BCG vaccine is one of the most widely used vaccines in the world, side effects are rare. The vaccine is contraindicated to people who are immune suppressed or pregnant. Due to the exclusion criteria and the high age of the participants, there should be no such contraindications among participants. The participants will presumably all have been vaccinated as children, which should lessen the risk of severe side effects. The vaccination can cause a slight transient pain at the moment of injection.

All participants will receive the IIV recommended for their age group by the Danish Medical Board. The amount of blood obtained (a maximum of 80 ml over a 42-day period) does not constitute a threat to participants.

During the course of the study, we might find increased blood sugar, an abnormal white blood cell distribution, or other indicators of underlying disease. In that case, the participant will be informed and referred to further examination unless she/he has indicated a wish not to be informed. There will be no economic compensation for participating in the trial.

Treatment, registration and reporting of side effects

As part of the study information, participants will be informed in writing and orally about the above-mentioned 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 about 4 weeks after the vaccine is given, and that in the longer term, a small ~5 mm diameter scar will appear where the vaccine was given. This kind of side effects will not be reported to the Danish Medicines

Agency.

In case of suspected side effects beyond the above mentioned, the participants will be asked to contact the investigator as soon as possible for registration, advice and, if necessary, clinical control and treatment.

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. Investigator will assess whether the SAE is likely to be related to the study vaccines when reporting to sponsor.

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 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 the last study vaccine is administered, regardless if this is BCG vaccine, Influenza vaccine or potentially COVID 19 booster

vaccine and 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.

Potential benefits for participants

The potential benefits include a strengthened antibody response to influenza vaccine and improved immune function.

Insurance

The study participants are fully insured according to the Patient Insurance Act.

FUNDING

Christine Stabell Benn (sponsor) has received a grant of 6,120,000 DKK from the Independent Research Fund Denmark for conducting studies on the effect of BCG vaccine and probiotics on the immune system of the elderly, including this study. The funds are placed on an SDU account (project id 21304). Please see budget for details. Neither sponsor nor any other member of the study group have any conflicts of interest concerning the study.

MAJOR MILESTONES

Application for Regional ethical committee submitted: June 2019.
Application for EutraCT submitted: June 2021.
Application for Danish Medicines Agency submitted: June 2021
Inclusion of first participant: September 2021
Inclusion of last participant: November 2021
End of follow-up last participant: 6 months post-randomisation or by latest 31st of May 2022
Paper writing and submission of papers: Until end of March 2023

PUBLICATION

Results will be uploaded in EudraCT (European Union Drug Regulating Authorities Clinical Trials Database) within one year of the completion of the trial. Data will be available at *clinicaltrialsregister.eu* hereafter. The trial will also be registered at www.ClinicalTrials.Gov. Positive as well as negative and inconclusive trial findings will be published. All publications based on

the trial will be aimed at international peer-reviewed medical journals.

PERSPECTIVES

If we can identify safe and low-cost interventions to improve vaccination responses and reduce morbidity in the elderly, we can improve the number of illness-free years to the segment of the population that is going to increase most significantly in numbers over future decades.

Provided we find positive effects of BCG, the next step will be to investigate the effect of providing yearly BCG. Noteworthy, boosting with BCG has shown promising potential with respect to mortality[18], bladder cancer[19], and type 1 diabetes[20].

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