Clinical Trial Protocol

Trial ID: ENFORCE

Title of Trial:

National Cohort Study of Effectiveness and Safety of SARS-CoV-2/Covid-19 vaccines (ENFORCE)

Danish National SARS-CoV-2 Vaccine Research Consortium

Investigational Medicinal Product:	Observational: multiple new SARS-CoV-2 vaccines approved for use by the European Medicines Agency
Sponsor, Funder:	
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Signatures:

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Contents

List of Abbreviations

ACE2	Angiotensin converting enzyme 2
AE	Adverse Event
AUH	Aarhus University Hospital
BP	Blood Pressure
CD4+	Cluster of differentiation 4
CD8+	Cluster of differentiation 4
CoV-2	Coronavirus 2
COVID-19	Corona Virus Disease 2019
CPR	Central Person Registry (Det Centrale Person register)
CRF	Case Report Form
DDV	Danish Vaccine Registry
DNB	Den Nationale Biobank
DSC	Data and statistical centre
DMA	Danish Medicines Agency
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
GCP	Good Clinical Practice
ICH	International Conference of Harmonisation
IMP	Investigational Medicinal Product
KMA	Klinisk Mikrobiologisk Afdeling
LPR3	National Patient Registry (Indberetning til Landspatientregisteret)
MD	Medical Doctor
MPNAT	Minimal Protective Neutralising Antibody Titre
NC	Nucleocapsid
NTD	N-terminal Domain
OD	Once Daily
RBD	The Receptor Binding Domain
S	Spike (protein)
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SARS	Severe Acute Respiratory Syndrome (caused by SARS-CoV-1)
SmPC	Summary of Product Characteristics
SSI	Statens Serum Institute
SUSAR	Suspected Unexpected Serious Adverse Reaction
T-cell	Type of leukocyte (white blood cell)

1. Protocol Synopsis / Summary

Title of trial:	**National cohort study of effectiveness and safety of SARS-CoV-2 vaccines**
Trial ID:	ENFORCE
Objectives:	The primary objective of the study is to assess effectiveness of citizens being vaccinated with one of SARS-CoV-2 vaccines the government has purchased, and the Danish Medicines Agency has approved for use in Denmark. The study will compare and predict the durability of the minimal protective titre afforded by each of the vaccines against COVID-19 through conducting comprehensive high-throughput SARS-CoV-2 antibody analyses and in-depth characterization of the vaccine-induced cellular immune response. The study has a number of secondary objectives. These can be classified in two main subgroups, those related to other assessments of effectiveness and those related to safety. The study will compare several outcomes between vaccine groups related to effectiveness.
Trial design:	National cohort study of effectiveness and safety of SARS-CoV-2 vaccines (ENFORCE) is an equivalence trial to evaluate the effectiveness and safety of multiple new SARS-CoV-2 vaccines approved for use in the EU, and which are being offered at participating units. The design is an open-labelled, non-randomised, parallel group, phase IV study with historical controls. Several sub-studies will be embedded within this master protocol addressing basic and translational research questions requiring additional sampling of biological material (under separate participant informed consent).
Trial population:	First phase will enrol 10,000 persons initiating vaccination (assuming 4 vaccines). If more vaccines become available additional 2,500 persons per vaccine will be included. Subsequent phases with larger sections of the population included may be implemented.
Methods:	Participants will have 6 study visits and be followed for 2 years after the first vaccination, which offers the participants an extra close follow up on vaccine effectiveness. Safety data will be collected at study visits until 3 months after the first vaccination. Research samples will be collected at each study visit during the two-year follow-up
Trial endpoints:	Primary outcome is the minimal protective neutralising antibody titre (MPNAT); i.e. the minimum level of neutralising antibodies sufficient to protect the person from becoming infected.
Trial medication:	The vaccines are not part of the study set-up and citizens offered inclusion in the study will be offered the vaccine regardless of their participation in the study. There is therefore a limited risk of participation in the study.
Trial schedule:	Planned first subject first visit: Q1 2021 Planned last subject enrolled: Q2 2021 Planned last subject last visit: Q2 2023 End of trial Q4 2023

2. Flowchart

Overview of visits and data collection

Visit number	**1***	**1b**	**2* / ****	**2b**	**3*****	**4**	**5**	**6**	
Day, week, month	**Day of first vaccination**	**For participants in Sub-study 2 only**	**Day of second vaccination**	**For participants in Sub-study 2 only**	**3 months after first vaccination**	**6 months after first vaccination**	**12 months after first vaccination**	**24 months after first vaccination (End of trial)**	**Historic case control arm (register data from 2018)**
Acceptable deviation (+/-) from date	**--14 days before vaccination**	**7 days (+/- 5 days) after first vaccination**	**-5 days before vaccination**	**7 days (+/- 5 days) after second vaccination**	**+/- 14 days**	**+/- 14 days**	**+/- 14 days**	**+/- 14 days**	
ELIGIBILITY & BASELINE DATA									**Na**
Informed consent[2]	X								Na
Baseline medical history[3,4]	X								Gender, age
Baseline medications[3,5]	X								Chronic diseases
STUDY INTERVENTION									**Na**
Type of vaccine[3]	X		X						Na
Vaccination Administration[3]	X		X						Na
STUDY PROCEDURES									Na
Provision of electronic/paper questionnaire to participant for collection of data until next visit[4]	X		X						Na
Collection and submission of questionnaire completed by participant covering period from last visit[5]			X		X	(X****)			Na
Interview on interim medical history/grade 3 and 4[3,6]			X		X	(X****)			Na
Grade 1 and 2 events present on the day of the visit			X		X	(X****)			Na
Interim medications[3]			X		X	(X****)			Na
SAE[3]	Reported within 7 days after notification								Na
Research sample storage (plasma & serum)	X	X	X	X		X	X	X	Na
Standard biochemistry	X	X							

Visit number	**1***	**1b**	**2* / ****	**2b**	**3*****	**4**	**5**	**6**	
Standard information on subject, incl. heritage/native country					From CPR				
Microbiological tests: performed and results					From MIBA registry				Yes
Full viral genome sequencing: SARS-CoV-2					From SSI as fasta file				Na
Vaccinations					From DDV registry				Yes
Deaths					From death registry				Yes
Hospital admission					From LPR3 registry				Yes
Biochemistry					LSP/Laba registry				yes
Pathology					LSP & Patobank				Yes
Prescriptions					From prescription registry				yes

* Visit must be before vaccination.
** If subject will only receive one vaccine shot, no Visit 2 will be performed
*** If the day of Visit 2 was 3 months (+/- 14 days) after 1st vaccine, no Visit 3 will be performed
**** Only If no Visit 3 was performed these data are collected at Visit 4
[1] Timing of visit 2 is determined by product insert of the optimal day of providing the booster of the specific vaccine use. It will probably be 3-4 weeks after first vaccination.
[2] Written version of signed informed consent stored locally, and documentation entered in electronic data capture system (e.g. REDCap).
[3] Data collected. Various components of the data captures made by study personnel outlined below – entered in electronic data capture system.
[4] All participants are sent an e-mail in the e-boks with a link to the electronic questionnaire. If, participant prefer paper the study staff hands out a paper form.
[5] The completed questionnaire is handed to the study site personnel – either electronically or paper. This information is uploaded electronically (either as a file (if collected electronically) or as a picture (if paper version; paper versions are entered centrally into the central database)).
[6] Grade 3 or 4 AEs (see text below on generic grading) since last visit and any grade at the time of the visit captured by an interview/data capture form and entered in electronic data capture system.

3 or more vaccinations *Addition of extra visits to flowchart at time of each dose*	**Additional dose(s)**	**Additional dose(s)**
Visit number	**X* and ****	**Xc*****
Day, week, month[1]	**Prior to vaccination**	**28 days after vaccination**
Acceptable deviation (+/-) from date	**(-14/+0 days)**	**(-8/+8 days)**
STUDY PROCEDURES		
Additional Informed Consent[2]	X	(X) *If no visit X was performed*
Provision of electronic/paper questionnaire to participant for collection of data until next visit[4]	X	
Collection and submission of questionnaire completed by participant covering period from last visit[5]		X
Interview on interim medical history/grade 3 and 4[3, 6]		X
Grade 1 and 2 events present on the day of the visit[3]		X
SAE[3]	Reported within 7 days after notification	
Research sample storage (plasma & serum)	X	X
Sub-study 1 if relevant	X	X

* Visit must be before booster and/or additional vaccination

** If the day of Visit X falls within 30 days before the window of a regular ENFORCE study visit (Month 6, Month 12 or Month 24) the regular ENFORCE visit will fall out of the schedule

*** If the day of Visit Xc falls within 30 days before the window of a regular ENFORCE study visit (Month 6, Month 12 or Month 24) that regular ENFORCE visit will fall out of the schedule

1. Timing of visit X and Xc depends on the date of the vaccination
2. Written version of signed additional informed consent stored locally, and documentation entered in the electronic data capture system
3. If participant has no visit X, additional informed consent is signed on Visit Xc
4. Data collected. Various components of the data captures made by study personnel outlined below – entered in the electronic data capture system
5. All participants are sent an e-mail in the e-boks with a link to the electronic questionnaire. If, participant prefer paper the study staff hands out a paper form
6. The completed questionnaire is handed to the study site personnel – either electronically or paper. This information is uploaded electronically (either as a file (if collected electronically) or as a picture (if paper version; paper versions are entered centrally into the central database))
7. Grade 3 or 4 AEs (see text in original protocol on generic grading) since last visit and any grade at the time of the visit captured by an interview/data capture form and entered in electronic data capture system.

3. Background / Rationale

3.1 Disease background and current treatment modalities

The Danish COVID-19 vaccination program will include several new SARS-CoV-2 vaccines, and the programme is expected to commence in January 2021, assuming all approvals etc. are on track. According to the national vaccine plan (Udrulning af vaccination mod Covid-19), vaccination will initially be offered to people in specified risk groups, and to selected key workers in the health, elderly and social care sectors. As the development of the vaccines has been performed during a limited time period it will be important to continue to gather more information about safety and effect as they are being rolled out.

The Danish National SARS-CoV-2 Vaccine Research Consortium will establish a national cohort study with 10,000 participants, which will be followed for 2 years after first vaccination to document effectiveness and safety of the vaccines. 2500 participants per vaccine will be enrolled. If more than 4 vaccines become available, an additional 2500 participants per vaccine will be enrolled.

There are two overall immune mechanisms of protection against COVID-19 – antibody mediated and cell-mediated immunity. Both mechanisms might contribute to protection in an individual, but it is also possible that one of these two mechanisms are sufficient in providing protection against symptomatic COVID-19. To determine the immunological footprint of the novel SARS-CoV-2 vaccines, the study will conduct both comprehensive high-throughput SARS-CoV-2 antibody analyses as well as in-depth characterization of the vaccine-induced cellular immune response. In so doing, we will also be able to compare and predict the durability of protection afforded by each of the vaccines against COVID-19.

3.2 Trial aim and rationale

The aim is to study primarily effectiveness as well as safety of citizens being vaccinated with one of the SARS-CoV2 vaccines being applied in the Danish COVID-19 vaccine programme.

Whereas ongoing phase III trials are reporting vaccine efficacy in defined study populations, the effectiveness of these vaccines – and in particular the durability hereof - once introduced into the general population is presently unknown, and the focus of intense research and public health interest.

Given the health system and data infrastructure in Denmark, the country is well suited to address this overarching aim.

Under separate patient informed consent, a sub-study cohort (Sub-study No.1) will be established including 250 participants (100 healthy and 150 “high-risk” individuals (as defined by SST in the national vaccination plan)) from each vaccine group (total 1000). Live cells (PBMCs) and PAX tubes (for transcriptomic analysis) will be collected for the participants in the sub-study. Several sub-studies will be embedded within this cohort addressing basic and translational research questions requiring additional sampling of biological material (see Appendix 3).

Under separate patient informed consent, a second sub-study cohort (Sub-study no. 2) will be established with subjects participating in this master protocol (See Appendix 6)

3.3 Third and subsequent vaccine doses irrespective of type of vaccine used

While ENFORCE was originally designed to monitor the immunological response to the standard recommended COVID-19 vaccine regimens at the time of inception over a two-year period (i.e. two doses), the scientific rational and subsequent implementation of the need of additional doses (3 or 4) to further boost/preserve immunity was clarified in the second half 2021. It is plausible, that during the study period, additional doses beyond those already administered will be required and hence used. ENFORCE intend to observe this evolving use of vaccines by the cohort included in the study, during the 2 year follow-up of the cohort, at time when participants are offered and have accepted such additional doses by the national health authorities. It is also possible that new vaccine technologies will be licensed and used as part of the national vaccine program. As such, ENFORCE will continue to assess the impact of use of such additional doses of existing vaccines and any

new types which may emerge. As this is an observation study it will not interfere with the participants choice of receiving such doses/types of vaccines.

Booster and/or additional vaccinations are rolled out as part of the Danish vaccination programme and ENFORCE has been asked by Sundhedsstyrelsen to consider extending the trial with additional study visits related to the introduction of booster and/or additional vaccination(s). This amendment describes the proposed extra study visits - one prior to and one 28 days after booster and/or additional vaccination. The purpose is to closely follow the participants' anti-body levels and record any additional safety issues related to the booster and/or additional vaccination. Currently, only vulnerable groups are offered booster and/or additional vaccination, hence not all ENFORCE participants will be invited to the additional study visits at this time. However, potentially booster and/or additional vaccination may be offered to all vaccinated people in Denmark, and thus cover all ENFORCE participants.

While booster and/or additional vaccinations are hoped to increase antibody levels in certain vulnerable populations that are known to be hyporesponsive to vaccination, the added immunological and clinical benefit as well as the potential side effects of booster and/or additional vaccination are unknown. Currently, there is very little knowledge globally on the safety of a third vaccination and it is highly relevant to generate new knowledge on this procedure. Thus, to address these important issues, this protocol amendment has been developed to the ENFORCE protocol.

The timing of the additional visit (X and Xc) depends on the date of the participant's booster and/or additional vaccination as Visit X is *prior* to booster and/or additional vaccination and Visit Xc 28 days *after* booster and/or additional vaccination:

- Visit X prior to the booster and/or additional vaccination (between 14 days and 30 minutes prior). Here participants will be asked to sign an additional informed consent before any additional trial activities, including blood samples, are performed. At visit X the ENFORCE standard blood samples are drawn. For participants participating in the Sub-study No. 1 additional blood samples are collected

- Visit Xc scheduled 28 days (-8/+8 days) after booster and/or additional vaccination. Here will be gathered safety information (AEs and SAEs) and ENFORCE standard blood samples are drawn. For participants participating in the Sub-study No. 1 additional blood samples are collected

All participants in ENFORCE will be informed via a letter in E-boks about the additional study visit(s) related to the booster and/or additional vaccination(s). Participants will be encouraged to book a study-visit prior to receiving the booster and/or additional vaccination. However, as it is anticipated that some participants will get a booster and/or additional vaccination without having a prior study visit we will, in addition, draw data from the national vaccine registry on all ENFORCE participants that have already received booster and/or additional vaccination; and those who did not have a Visit X will be contacted by study staff and invited to the additional study visit Xc 28 days after vaccination.

3.4 Benefit-risk assessments and ethical considerations

Participants will be followed for 2 years after the first vaccination, which offers the participants an extra close follow up on vaccine effectiveness. The participants will have to present to donate blood samples at 6 timepoints. The vaccines are not part of the study set-up and citizens offered inclusion in the study will be offered the vaccine regardless of their participation in the study. There is therefore a limited risk of participation in the study.

A historical control group (10 to 1) of age, sex and geographic matched control patients alive on 1st January 2018 will be used to establish baseline incidence of safety-related outcomes from registries.

4. Hypothesis

The study is an equivalence trial and it will compare, between vaccines, the fraction of study participants with minimal protective neutralising antibody titre (MPNAT) at various timepoints after initiation of vaccination
It is hypothesised that the fraction of study participants with MPNAT will be equivalent between the vaccines over time i.e. there is no difference between the vaccines. While true equivalence cannot be ascertained, we hypothesis that the vaccines will be within a clinically acceptable margin such that neither one can be considered inferior or superior.

5. Objectives

5.1 Primary objective

The primary objective of the study is to assess effectiveness of citizens being vaccinated with one of SARS-CoV2 vaccines the government has purchased, and the Danish Medicines Agency has approved for use in Denmark. The study will compare and predict the durability of the minimal protective titre afforded by each of the vaccines against COVID-19 through conducting comprehensive high-throughput SARS-CoV-2 antibody analyses and in-depth characterization of the vaccine-induced cellular immune response.

5.1.1. Primary endpoint

Primary outcome is the minimal protective neutralising antibody titre (MPNAT); i.e. the minimum level of neutralising antibodies sufficient to protect the person from becoming infected.
MPNAT will be measured via profiling of antibodies against SARS-CoV-2 Spike epitopes performed at each visit until month 24. We will use two different large-scale methods. ELISA detection of total serum Ig to the Receptor Binding Domain (Wantai) and a multiantigen serological test including both the N-terminal Domain (NTD), The Receptor Binding Domain (RBD), the complete Spike (S) protein and the Nucleocapsid (NC) protein as antigens (from Mesoscale). Additionally, an ACE2 competition assay will be used to score the receptor blocking potential of antibodies raised by vaccination (Mesoscale). These assays are high-throughput and will be applied to stored plasma samples from enrolled participants (for details, see Sub-study No. 1 in Appendix 3). In addition, other assays for quantifying SARS-CoV-2 vaccine responses and immunity may be added if they provide additional scientific insight.

The exact value of the MPNAT is currently not precisely defined but is expected to be documented within short periods of time based on analyses across the ongoing phase III trials, associating titre levels with risk of breakthrough infection in the actively vaccinated group. Based on available evidence of the serology vaccine response from phase I and II studies of the vaccines with documented high vaccine efficacy (i.e. the two mRNA vaccines), the MPNAT that neutralises 80% of viral replication *in vitro* is projected to be maximally 300, but may likely be lower than that.

As the exact value of the MPNAT remains to be determined, and until that time point has arisen, *a priori* (i.e. while remaining blinded to the actually obtained data) of the actually defined cut-offs in neutralising titres that reasonable can serve as proxy for the MPNAT will be recommended by an expert advisory panel, and endorsed by the study leadership.

5.2 Secondary objectives

The study has a number of secondary objectives. These can be classified in two main subgroups, those related to other assessments of effectiveness and those related to safety.

In relation to effectiveness, the following outcome will be compared between vaccine groups:

- Breakthrough infections throughout the 24-month follow-up period.
    - Participants are encouraged to seek testing at one of the national testing sites in situations where the person suspects she/he may have ongoing SARS-CoV-2 infection (symptoms or close contact). If confirmed, the study relies on central standard procedures for viral sequencing via the set-up coordinated by SSI and will request that the data file is electronically transferred to the central database. If obtained, full genome viral sequences will be compared to circulating viral strains obtained from individuals not part of the initial vaccine roll-out. Discovery of genetic variant differences in these two populations will be investigated through bioinformatic sieve analysis.

  - Breakthrough infections are also monitored by the longitudinal analyses of non-vaccine induced SARS-CoV-2 antibodies (e.g. nucleocapsid antibodies)

- A series of more detailed immunological assessment in subgroups of participants of markers of cellular immunity (see Sub-Study No. 1, appendix 3)

In relation to safety, the following outcome will be compared between vaccine groups (within first 7 days after each vaccination, and through three to six months after initial vaccination):

- Participants with local and systemic reactions to vaccination
  - This participant centred outcome will be ascertained using a symptoms form that each participant completes 7 and 14 days after the vaccination
    .
- Grade 3 and 4 adverse events and serious adverse events
  - This will be ascertained and reported by study-affiliated staff. Primary safety outcomes are any grade 3 or 4 events observed within the first three to six months after the initial vaccination. These outcomes are retrieved by study staff in an interview form at two time points (visit 2, visit 3 or visit 4, if no visit 3 performed). SAE are ascertained within first three to six month after study entry and reported within 7 days of sites being notified.

- Grade 1 and 2 events
  - This will be ascertained by study-affiliated staff as present on the specific day of visit 2, visit 3. or visit 4, if no visit 3 performed. Here study staff capture this in the interview form. Stored plasma may be used to assess markers of host response to the vaccination.

A historical control group (10 to 1) of age, sex and geographic matched control patients alive on 1st January 2018 will be used to establish baseline incidence of outcomes from registries.

6. Trial design

6.1 Summary of trial design

This is an open-labelled, non-randomised, parallel group, phase IV study with historical controls.

The study will collect clinical data related to effectiveness and safety from 2.500 participants for each introduced SARS-CoV-2 vaccine, in whom a well curated and detailed research biobank of biological materials will also be constructed. All data will be collated in one single central database of multiuser analysis.

The study population consists of consecutive consenting persons invited to be vaccinated at participating units throughout Denmark.

The regimen is any of the vaccines the government has purchased, and the European Medicines Agency/European Commission has authorised for use, and which are being offered at participating units.

Type of vaccine is the primary stratification parameter. The equivalence trial will, between vaccines, compare the fraction of study participants with minimal protective neutralising antibody titre at various timepoints after initiation of vaccination.

Additionally, subgroups will be assessed depending on demographic parameters (age, gender, ethnic background, location of facility offering the vaccination), anti-SARS-CoV-2 antibody status at time of vaccination, and existing chronic co-morbidities at time of vaccination.

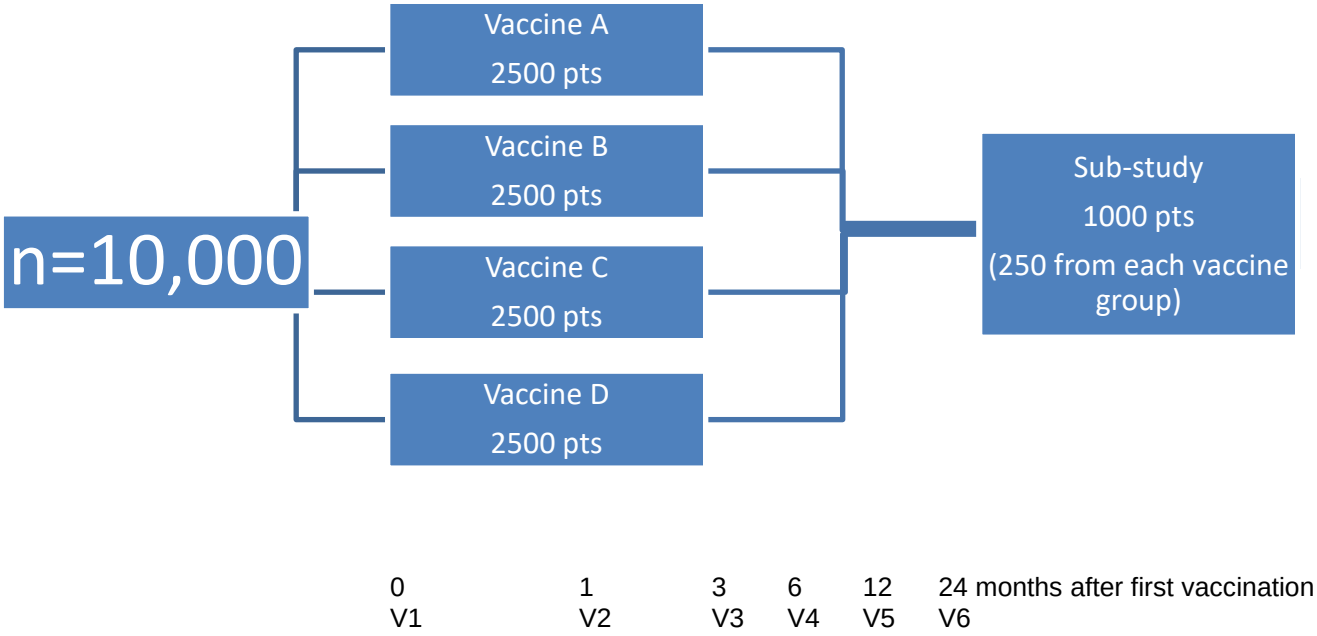
Enrolment will occur in phases. First phase will constitute a sample size of 10,000 persons initiating vaccination (assuming 4 vaccine groups of 2500 persons). Subsequent phases with larger sections of the population included may be implemented, providing a sound science justification, satisfactory conduct of first phase, and that additional funding has been secured. Also, if more than 4 vaccines will be applied, additional 2500 persons per vaccine can be included.

With an equivalence margin set at δ=+/-5%, a sample size of 2,500 per group will provide sufficient power to ascertain equivalence between two vaccines with ≥90% certainty.

For all study participants, whole blood sample is collected at all 6 study visits totaling 60.000 samplings. All study participants are asked to reply to an interview form at two time points (visit 2, visit 3 or visit 4, if no visit 3 performed) to retrieve any grade 3 or 4 events within the first three to six months after the initial vaccination. Additional safety outcomes, including hospitalizations and deaths, are retrieved electronically from national registries as outlined in flowchart page 7. Finally, stored plasma may be used to assess markers of host response to the vaccination.

Additional biological samples (250 per vaccine) will be collected in a fraction of the cohort as part of consent to participation in the individual sub-study cohorts.

Figure 1 - Trial design



If additional vaccines become available, additional 2500 persons per vaccine and 250 in the individual sub-studies can be included.

6.2 Trial Schedule

Phase 1:

Planned first subject first visit:	Q1 2021
Planned last subject enrolled	Q2 2021
Planned last subject last visit:	Q2 2023
End of trial	Q3 2023

6.3 Discussion of Design

With the planned fast roll-out of the new SARS-CoV-2 vaccines it is not possible to design a study with a non-vaccinated control group. However, a matched control group will be established based on register data.

The vaccines studied will be the ones available and used at the participating sites.

7. Trial population

The study population consists of consecutive consenting persons invited to be vaccinated at 20- 50 participating units throughout Denmark. A targeted effort will be made to enrol a high proportion of participants who are considered at 'high-risk' of COVID-19 (as defined by SST in the national vaccination plan).

Citizens booking time for vaccination (e.g. via vacciner.dk) will have the possibility via a new link to get information of the ENFORCE study.
Citizens who are offered the invitation for vaccination via their e-boks and citizens who already have booked time for vaccination can afterwards be informed of the study per e-mail or letter (normal mail or e-boks). Study staff will be present at vaccination centres to inform citizens of the study.

Information about the ENFORCE study will also be advertised through public channels such as newspaper-adds, Facebook- and Instagram-posts, posters and flyers in hospitals and general practitioners' offices, and via intranet and email-lists to hospital- and health staff. Study visits can be booked electronically or via phone at study sites and should occur from 14 days before vaccination visit is scheduled until 30 minutes before vaccination visit.

An Information video with the Investigators will be published via the information channels at the hospitals. In the information video one or more Investigators and/or project nurses will participate to shortly inform of the background and purposes of the study, who and how many can participate, what it involves to participate and how to get in contact with the study staff, if interested in the study and how the results will be used.

Citizens/patients who are signed up for vaccination by their hospital department or private specialist can be contacted directly by the hospital department or the private specialist via e-mail or via post/e-boks and be informed of the possibility of participating in the study. If, an already regular telephone contact is planned with the patient, information of the trial may be given per telephone.

Subjects meeting all the inclusion criteria listed and none of the exclusion criteria will be considered eligible for the trial.

Eligible subjects will be informed and offered entry into the individual Sub-studies under separate consents.

7.1 Inclusion criteria

1. Written informed consent obtained before any trial related procedures are performed
2. Male or female eligible for SARS-CoV-2 immunization (as defined by SST in the national vaccination plan)
3. The subject must be willing and able to comply with trial protocol (re-visits and biological samples)

7.2 Exclusion criteria

1. Male and female under the age of 18
2. Any subgroup of individuals for which the vaccines are contra-indicated
3. Previous SARS-CoV-2 vaccination

8. Procedures

8.1 Visit Schedule

In the table below, the procedures that should be performed at each visit are described. The procedures are listed in the chronological order in which they should preferably be performed:

Visit ID	Procedures to be performed at the visit
Invitation phase	Citizens who have shown interest in participating in the study will be scheduled for an enrolment visit.
Visit 1 (Enrolment Visit) **(–14 days and until 10 minutes before first vaccination)**	Provide information and answer questions about the ENFORCE study Obtain written informed consent for the trial before any other trial procedures are performed *Inform and offer entry into the individual Sub-studies under separate consents (selected sites only)* Obtain CPR-number, date and venue of vaccination Record relevant medical history and concomitant medication (incl., existing chronic co-morbidities at time of vaccination) Assess compliance with inclusion and exclusion criteria Collect whole blood in 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL “dry glass” (for serum isolation) Give instructions to participants on the symptoms form that shall be filled out at home *For participants consenting to the Sub-study No. 1 cohort collect blood samples.* *Schedule date for next study visit at time of second vaccination (-5 days before).*
(Day of first vaccination) ***[not part of this study]***	*Vaccination with the COVID-19 vaccination available at local vaccination centre*
Visit 1b **(7 days (+/- 2 days) after first vaccination)**	For participants who has consented to Sub-study No. 2 collect blood samples
Visit 2 **(– 5 days before until 30 minutes before second vaccination)**	Record any signs and symptoms Collect whole blood in 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL “dry glass” (for serum isolation) *For participants who has consented to the Sub-study No. 1 collect blood samples* Interview participant and assess grade 3 and 4 AEs occurring since the day of vaccination, as well as grade 1 and 2 events present on day of visit Collect symptoms form filled out by subjects at home Schedule date for next visit

Visit ID	Procedures to be performed at the visit
(Day of second vaccination) ***[not part of this study]***	*2nd COVID-19 vaccination available at local vaccination centre, if applicable*
Visit 2b **(7 days (+/- 2 days) after second vaccination)**	For participants who has consented to Sub-study No. 2 collect blood samples
Visit 3 **(3 months after first vaccination)**	If the day of Visit 2 was 3 months (+/- 14 days) after 1st vaccine, no Visit 3 will be performed Interview participant and assess grade 3 and 4 AEs occurring since the last visit, as well as grade 1 and 2 events present on day of visit Collect symptoms form filled out by subjects at home Collect whole blood 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL “dry glass” (for serum isolation) *For participants participating in the Sub-study No. 1 collect blood samples* Schedule date for next visit
Visit 4 **(6 months after first vaccination)**	If no visit 3 was performed, interview participant and assess grade 3 and 4 AEs occurring since the last visit, as well as grade 1 and 2 events present on day of visit Collect symptoms form filled out by subjects at home Collect whole blood in 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL “dry glass” (for serum isolation) *For participants participating in the Sub-study No. 1 collect blood samples* Schedule date for next visit
Visit 5 **(12 months after first vaccination)**	Collect whole blood in 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL “dry glass” (for serum isolation) *For participants participating in the Sub-study No. 1 collect blood samples* Schedule date for next visit
Visit 6 **End of trial** **(24 months after first vaccination)**	Collect whole blood in 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL “dry glass” (for serum isolation) *For participants participating in the Sub-study No. 1 collect blood samples* Schedule date for a telephone follow-up contact, if applicable

Addition of the Visit X and Visit Xc related to the third vaccine or booster/additional vaccinations to the visit schedule table with description of procedures that should be performed:

Visit ID	Procedures to be performed at the visit
Invitation phase	All participants in ENFORCE will be contacted via e-boks with information about the additional study visit (Visit X and Visit Xc) and invited to book a visit prior to booster/ additional vaccination Participants in ENFORCE that have received a booster/ additional vaccination, but not had a visit X, will be contacted by study staff and invited to an additional study visit (Visit Xc) 28 days after booster/ additional vaccination
Visit X (pre-Vaccination Visit) **(-14 days and until 30 minutes before vaccination)**	Collect whole blood in 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL "dry glass" (for serum isolation) Give instructions to participants on the symptoms form (XA and XB) that shall be filled out at home *For participants consenting to the Sub-study No. 1 cohort collect blood samples* Schedule date for next study visit 28 days after booster/additional vaccination (-8/+8 days)
Visit Xc **28 days (- 8/+8 days) after vaccination**	Collect whole blood in 1x 6 mL EDTA tube (for plasma isolation) and 1x 6 mL "dry glass" (for serum isolation) *For participants who has consented to the Sub-study No. 1 collect blood samples* Interview participant and assess grade 3 and 4 AEs occurring since the day of booster/ additional vaccination, as well as grade 1 and 2 events present on day of visit Collect the day 1-7 and day 8-14 symptom forms filled out by subjects at home

8.2 Trial Procedures

This section outlines the trial procedures that will be performed during the trial. For further details on the specific timing of the procedures please refer to the visit schedule in section 8.1.

The tasks listed below must be performed by investigator/study nurse or other trained study personnel.

- Obtainment of informed consent
- Evaluation of in- and exclusion criteria
- Extraction of whole blood (+ *For subjects enrolled in Sub-study No. 1 collect live cells (PBMCs) and PAX tubes)*
- Conduct participant interview to assess grade 3 and 4 AEs/SAEs, and capture grade 1 and 2 on the day of the visit (visit 2, visit 3 or visit 4, if no visit 3 performed)
- Assessment of other laboratory results

8.3 Informed Consent

The written informed consent must be obtained (i.e. signed and dated by the subject) before any trial activities are performed.

Each subject must be informed that participation in the trial is voluntary and that he/she may withdraw from the trial at any time during the course of the trial and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Subjects meeting the inclusion criteria for the trial will be give verbal and written information about the trial. The subject will be given time to ask questions and allowed sufficient time to consider the trial before deciding whether to participate.

If subjects so desire, they may make a later appointment with the site investigator after having reviewed the written information about the trial, at which they can meet and discuss participation in the trial. Such a meeting must take place in an undisturbed room and the subject may bring a family member or friend to aid the decision-making. After this meeting the subject may deliberate on trial participation for up to seven days.

The subjects will be informed of any potential risks associated with the trial.

It is the responsibility of the study staff to obtain the written informed consent from the subject.

At time of enrolment, all participants are also offered entry into one or more available sub-studies under separate consent.

Further, consent to the participation in the trial, will give sponsor, principal investigator and GCP representatives access to patient records for clinically relevant information regarding eventual hospitalisations, necessary for the conduct of the trial and in relation to quality control and required monitoring.

After completion of trial, all participating subjects will be informed of the overall results.

Consent for long-term storage of serum and plasma samples in a Biobank for future research

When the subjects are asked to consent to the participation in the trial, they will be asked specifically if they can accept the storage of their samples within a Biobank for future research. The answer to this question will be recorded on an addendum to the informed consent form, the Consent Form for Retention of Serum and Plasma Samples for Future Research, as well as on the Case Report Form (CRF). Subjects are able to participate in the trial without giving consent to long-term storage of plasma and serum samples after the end of this study.

8.4 Subject ID number

All subjects enrolled must be identifiable throughout the trial.

8.5 Demography

The following data will be recorded:

Study site information: date of first visit, Clinical site

Demography and basic information: date of birth, gender, heritage/country of origin

Vaccination status: Date, place (venue) and type of COVID-19 vaccination incl. Batch no.

8.6 Medical history and Concomitant disease

Medical history: In the 12 months prior to and including inclusion, did the participants experience any of the following (including any diagnosis that required regular follow-up, medicalisation, or hospitalisation within the past 12 months: Asthma, Cerebrovascular event (thrombotic or haemorrhagic), Chronic obstructive pulmonary disease (OCPD), COVID-19, Diabetes Mellitus requiring medication, Heart failure, Hepatic impairment, HIV, Hypertension requiring medication, Immunosuppressive disorder other than HIV, Malignancy (active or receiving treatment), Myocardial infarction (MI) or other acute coronary syndrome or Renal impairment

Concomitant Medications; Is the participant currently (within 24 hours) taking any of the following medications? Antirejection medicine after solid or stem cell transplant; Immune modulators, Corticosteroids (>10 mg of prednisone or equivalent), Treatment with biological medicine to treat autoimmune disease or cancer.

Information from subjects' medical records regarding co-morbidities and intercurrent disease will be recorded at baseline and throughout the course of the trial.

8.7 Laboratory Tests

For all study participants 12 mL whole blood is collected at all study visits. Whole blood will be taken using standard venepuncture techniques and will be draw of on 6 mL EDTA Plasma and one 6 mL vacutainer Serum (tørglas).

A total of 72 mL whole blood will be collected per subject over a period of 24 months.

For participants receiving booster/additional vaccination(s) additional 24 mL whole blood will be collected per booster/additional vaccination.

Research Biobank:

This material will be transported directly to Den Nationale Biobank (DNB) at SSI. The shipment will go together with regular samples collected transported daily from KMA to DNB and SSI.

At DNB samples are processed (including centrifugation at 500g for 10 min) and aliquoted in 3 x 1 mL cryovials (3 vial serum and 3 vial plasma). 100 µL serum in deep-well plates will be shipped from SSI to Department of Infectious Diseases at AUH.

AUH conducts full Spike protein serology and ACE2 competition serology (Mesoscale Diagnostics).

The following laboratory variables will be measured:

As part of the process 150 µL is transferred to SSI who will perform real-time SARS-CoV-2 serology (using the WANTAI ELISA based assay).

Biobank for future research:

For participants consenting to this, remaining samples will be stored in DNB for future research.

Sub-study No. 1

For participants consenting to participate in the sub-study evaluating T cell immunity Peripheral Blood Mononuclear Cells (PBMC) extra blood samples will be collected at all study visits.

Additional three CPT- Citrate vacutainers (each 8 mL blood) and one PAXgene tube (2 mL blood) will be drawn.

This material is shipped directly to Department of Infectious Diseases at AUH for processing.

Lab values:

- PBMCs will be purified from the three CPT-vacutainers. This process involves centrifugation at 1700g for 20 min, harvest of PBMCs by aspiration. An additional wash step involving centrifugation at 500 g for 15 min. Purified PBMCs are then counted and SARS-CoV-2 specific T cell immunity will be evaluated via the Activation Induced Marker (AIM) Assay using flow cytometry.

- Plasma from the CPT tubes will be harvested, and the Mesoscale Diagnostic Multiantigen serology Assay will be performed.

Research Biobank:
The remaining PBMC (expected 10 million aliquot) will be stored in a research Biobank located at -170 C in Liquid N_2 contained at AUH. The collected PAXgene tube is inverted and stored in the Biobank at -80 C.

Biobank for future research:
After the study, for participants consenting to this, remaining samples will be stored in DNB for future research. All participants who have given consent to participate in Sub-study No. 1, will at their first coming visit after 01 November 2021 have the oral information of this Biobank of the remaining samples, and hereafter give written consent, if participation is accepted.

Sub-study No. 2

For participants consenting to participate in the sub-study No. 2 evaluating immunological reaction which may involve platelet activation and clinical disease, extra blood samples will be collected.

For participants consenting to participate in the sub-study No. 2 these extra blood samples will be collected at two extra visits 7 days (+/- 5 days) after the 1st and 2nd SARS-CoV-2 vaccination, but maybe needed to be repeated depending on the issue of concern. A maximum of 80 ml blood will be drawn at each visit, but no more than 240 ml. will be drawn in total.

Lab values:

The laboratory assessments made in conjunction with implementing this sub-study will need to be adaptive depending on the issue(s) of concern. The three main domains of sample collection are:

i. biochemistry incl. haematology performed at local routine lab.

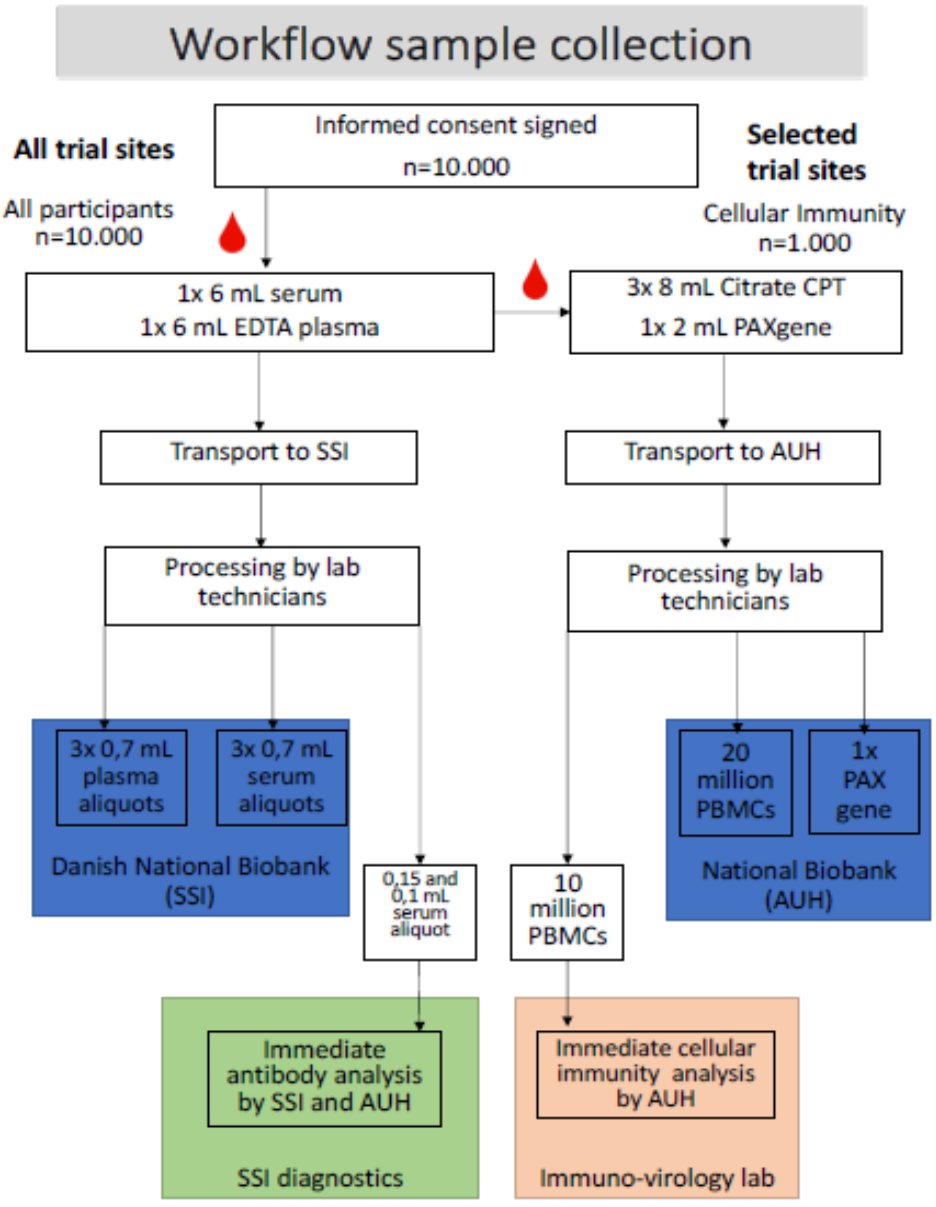
ii. plasma and serum for additional analyses in batches; live cells (PBMCs) for characterization of cell phenotype and activation status

iii. PAX tubes for transcriptomic analysis using RNAseq or similar methodology. The purpose of the transcriptomic analysis is to determine the impact of vaccination on epigenetic regulation, up- or downregulation of signalizing pathways (e.g. pro-inflammatory pathways, coagulatory pathways, vascular adhesion pathways), and immunologic phenotyping.
No genetic analysis will be performed. Transcriptomic data will not be used for genetic analysis either.

This material is shipped directly to Department of Infectious Diseases at AUH for processing.

Research Biobank:
The remaining PBMC will be stored in a research Biobank located at -170 C in Liquid N_2 contained at AUH. The collected PAXgene tube is inverted and stored in the Biobank at -80 C.

Biobank for future research:
After the study, for participants consenting to this, remaining samples will be stored in DNB for future research.



8.8 Participant Questionnaire/Symptom Form

All subjects will be issued a questionnaire/Symptom Form at the inclusion-visit. The subjects are asked to fill in the form on day 7 and day 14 after the first and the second vaccination. Subjects are also asked to fill in the forms after a third vaccination, booster and or additional vaccination(s). In the Symptom Form the subjects report and grade the following symptoms as mild, moderate or severe:

- Systemic reactions: Arthralgia, fatigue, fever, chills, headache, myalgia, nausea and diarrhea
- Local reactions: Pain, redness, swelling

8.9 Interview form

On visit 2, visit 3 or visit 4, if no visit 3 performed, study staff interview the participants to assess safety – see section 9 below.

Study staff also interview participants to assess safety at Visit Xc after the third, booster and or additional vaccination(s)

9. Assessment of safety

Primary safety outcomes are any grade 3 or 4 events observed within the first three months after the initial vaccination. This outcome is retrieved by study staff at two time points (visit 2, visit 3 or visit 4, if no visit 3 performed) via an interview form. At these two visits, grade 1 and 2 events present on that day are also captured and assessed. SAE are ascertained within first three month after study entry and reported within 24 hours of sites being notified. It is the responsibility of the sponsor to ensure that this data is retrieved as part of safety monitoring. Finally, stored plasma may be used to assess markers of host response to the vaccination.

A historical control group (10 to 1) of age, sex and geographic matched control patients alive on 1st January 2018 will be used to establish baseline incidence of outcomes from registries.

Within the first three months after vaccination and one month after booster and/or additional vaccination(s), information about Adverse Events (AEs), whether reported by the subject, discovered by the study staff at the interview, detected through physical examination, laboratory test or other means, must be collected and recorded on the AE form and followed up as appropriate.

Evaluation of AEs including severity, causality, outcome and seriousness assessments must be performed by a study investigator.

Any AE occurring from the time the informed consent was signed by the subject and until visit 3 or visit 4, if no visit 3 performed must be recorded and reported on an AE page in the CRF.

Standardised report forms for AEs and SAEs will be provided as part of the CRF (or eCRF).

Symptoms recorded by the subjects during the 7 to 14 days after vaccination on the symptom form are the following:

- Local symptoms (at the injection site): Redness, swelling and tenderness
- Other symptoms: Muscles pains, joint pains, fatigue, fever, chills, headache and nausea

None of these should be recorded as AEs, unless events are graded by the site staff as grade 3 or 4 at the following visit.

9.1 Definitions – Adverse Event (AE)

An AE is any untoward medical occurrence in a subject administered a medicinal product/device and which does not necessarily have a causal relationship with this treatment.

The following events should <u>not</u> be recorded as AEs:

- A pre-planned procedure, e.g. a surgical intervention, unless the condition for which the procedure was planned has worsened since the informed consent form was signed.
- Pre-existing conditions documented as medical history. Any worsening in severity or frequency of a pre-existing condition during the clinical trial period must be regarded as an AE.

9.2 Definitions – Serious Adverse Event (SAE) / Serious Adverse Reaction (SAR)

A serious adverse event/reaction is an experience that at any dose results in any of the following:

- Results in death
- Is life-threatening - this refers to an event in which the subject was at risk of death at the time of the event
- Requires in-subject hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is judged medical important (this refers to an event that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed above)

9.3 Assessments of AEs and SAEs

Grading of Severity:

The severity of an AE/SAE is a clinical observation assessed by the investigator using the following definitions:

For the grading of AE's the DAIDS toxicity table: (https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables) will be used. If event is not identified, the generic AE grading table below will be used.

GENERIC AE GRADING SCALE

- Grade 1 Events causing no or minimal interference with usual social and functional activities, and NOT raising a concern, and NOT requiring a medical intervention/ therapy.
- Grade 2 Events causing greater than minimal interference with usual social and functional activities; some assistance may be needed; no or minimal medical intervention/therapy required.
- Grade 3 Events causing inability to perform usual social and functional activities; some assistance usually required; medical intervention/therapy required.
- Grade 4 Events causing inability to perform basic self-care functions; medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
- Grade 5 Events resulting in death

Causality / Causal Relationship to IMP (vaccines):

The following terms and definitions are used when assessing the relationship between an AE/SAE and the relevant trial product (vaccine):

- Unlikely: the event is most likely related to aetiology other than the trial product
- Probable: good reason for sufficient documentation to assume a causal relationship

Final outcome:

The outcome of an AE/SAE is assessed by the Investigator using the following definitions:

- Recovered/resolved: Fully recovered or has returned to baseline
- Recovered with sequelae: As a result of the AE the subject suffered persistent and significant disability/incapacity
- Not solved
- Not recovered: The condition has not returned to baseline, however symptoms may have improved
- Fatal: Event that results in death
- Unknown: The outcome is unknown. This term should only be used when no other definition is possible e.g. the subject is lost to follow-up

9.4 Reporting

Serious Adverse Events:

SAEs must be reported immediately reported on the SAE eCRF (REDCap) within 24 hours of site awareness. Any follow-up data must be detailed in a subsequent SAE Form in due time.

The initial SAE report must contain as much information as possible including relevant CRF pages (e.g. medical history, concomitant medication) and must be provided via the e-CRF system. If the reporter does not have access to the e-CRF, e-mail may be used.

Study responsible/doctor at site must review the SAE report and assess causality and relation to the vaccines.
When an SAE form is filled out the system (REDCap) immediately sends an automated alert to the Sponsor's project email.
Sponsor study staff reviews all incoming SAE reports for quality and completeness of data.
Sponsor's Medical Officer reviews all SAE's and assesses causality and relation to the vaccines.

Suspected unexpected serious adverse reaction (SUSAR):

An unexpected serious adverse reaction with source or severity is not consistent with information in the current Summary of Product Characteristics (SmPC).

It is the responsibility of the Sponsor to determine whether a reported SAR is a SUSAR. A SUSAR resulting in death or is life threatening must be reported to the Danish Health and Medicines Authority and the Committee System on Biomedical Research Ethics within 7 days after the Sponsor becomes aware of the reaction. Within 8 days after initial reporting, the sponsor must report all relevant information concerning follow-up of the reaction. Any other SUSAR must be reported to the above mentioned agencies within 15 days after the Sponsor becomes aware the reaction.

10. Data handling / Data Management

The Data and Statistical centre (DSC) will serve as sponsor and is responsible for creating a central database, develop electronic case-report forms for data capture into the database, develop data quality systems to ensure optimal data collection, train and interact with each of the regional coordinators responsible for facilities within the region entering data, develop standardized reports of descriptive data, and oversee the statistical analyses of data. The central database will be electronically linked to and download data in real time from the Danish Vaccine Registry (DDV), LPR3, etc.

A central database will be established. Real-time access of data from registries to the data base (incl. from LPR3, VVR, Prescription registry, contacts to primary care etc)) is technically possible but will require political and legal clarification.

The project will be submitted to the Region H Videncenteret for dataanmeldelser as the data processor and to SDS for the registry data access.

Region H and Region Midt share the data-responsibility for this project, therefore the project also will be registered on Region Midt's internal list of research projects, as it is a matter of joint data responsibility.

The Data Protection Act (Databeskyttelsesloven) and the Data Protection Regulation (Databeskyttelsesforordningen) are complied with the trial, including the Data Protection Regulation, Chapter V. The Data Protection Act and the Data Protection Regulation are complied with when processing personal data in the trial.
Samples from the Biobank and Sub-Study Biobank (both for future research) can, in specific cases, be send for analysis in third countries. which has been accepted by consent from the individual participant. Additional analysis will only be performed after approval by the Committee System on Biomedical Research Ethics.

Data will be stored for 25 years, after which they will be transferred to "Dansk Data Arkiv" in an anonymised format.

10.1 Case Report Form

An electronic CRF is provided and all data related to the trial will be recorded in here and provide the basis for a central database.

The e-CRF is to be completed by the investigator at the time of the subject's study visit so that it always reflects the latest observations for the subject.
At the subject's final visit, the e-CRF should be verified and signed off by the responsible investigator at the site.

10.2 Subject Interviews

Participants are interviewed by study staff at two time points (visit 2, 3 or visit 4, if no visit 3 performed) to record any adverse events.

Information about, e.g. safety and medication, may be transcribed by the investigator to the relevant CRF page.

This is also applicable to visit Xc after the third vaccination and the follow-up vaccine(s).

10.3 Questionnaire/Symptom Form

Participants are requested to fill out a questionnaire/Symptom Form electronically or on paper form. The forms are collected and reviewed by the investigator. There will be no other source documentation for these data than the electronic/paper questionnaire.

10.4 Laboratory Data

- Whole blood will be collected at all study visits totaling 60.000 samplings (10,000 participants). Plasma will be collected from EDTA-tubes following centrifugation. Plasma will be aliquoted (3x 1 ml) and stored at -80 C. Serum will be collected following centrifugation and stored in 3x 1 ml aliquot at -80 C
- Antibodies in serum from all enrolled participants will be quantified from inclusion to month 24 using the standard Wantai ELISA method and the multi-antigen test (Mesoscale). The Mesoscale multiantigen serological test includes both the N-terminal Domain (NTD), The Receptor Binding Domain (RBD), the complete Spike (S) protein and the Nucleocapsid (NC) protein as antigens. Other assays for quantifying SARS-CoV-2 vaccine responses and immunity may be added during the course of the study. This will provide reference serology measure on all participants and can provide robust longitudinal immunity data.

10.5 For participants with break-through infection

For participants with break-through infection, the study relies on:

1. Central standard procedures for viral sequencing via the set-up coordinated by SSI and will request that the data file is electronically transferred to the central database
2. Longitudinal monitoring of SARS-CoV-2 vaccine- and non-vaccine induced antibodies

Real-time access of data needed for the study from registries to the central data base (incl from LPR3, VVR, Prescription registry, contacts to primary care etc.)) will be applied from the designated authorities (eg. ethical committee system/CVK/SDS) and adhere to all requirements for data protection. Ideally it will include data from:

- Standard information on subject, incl. heritage/native country from CPR
- Microbiological tests: performed and results from MIBA registry
- Full viral genome sequencing: SARS-CoV-2 from SSI as fasta file
- Vaccinations from DDV registry
- Deaths from death registry
- Hospital admission from LPR3 registry
- Biochemistry from LSP/Laba registry
- Pathology from LSP & Patobank
- Prescriptions from prescription registry

11. Statistical evaluation

11.1 In general

Per-protocol statistical analysis will be conducted by the Investigators.

Before analysing the cleaned dataset, a thorough plan for statistical analysis will be elaborated and accepted by the statistician and investigators.

All data will be described including data-incompleteness as well as reasons for data-incompleteness. Data will be analysed blinded by the principal investigator. Any changes to the statistical analysis plan will be described in any future publications.

As part of the initial phase of the study, a determination of the required MPNAT level to maintain immunity with respect to SARS-CoV-2 will be performed. This will define the binary outcome (MPNAT level sufficiently high, yes/no) for the main analysis comparing effectiveness of the four different vaccines.

The main analysis will be adjusted for age and sex, as well as covariates which are found to be univariately associated with the outcome. This is not accounted for in the justification for the sample size, as their identity is unknown, and will in any case only lead to a slightly lower precision in the comparisons [1]

The main aim of the analysis is to establish equivalence of all four vaccines, i.e. no particular vaccine leads to a lower titre level than any of the three others. A clinically relevant equivalence margin (δ) will be defined prior to the commencing of the study.

11.2 Justification of sample size/power calculation

A total of 2,500 persons per vaccine will be included. Table 1 shows the minimum equivalence margin (δ) that can be attained with 90% certainty when comparing two vaccine groups for varying vaccine efficacy levels (% achieving MPNAT) and sample sizes. Computations are based on simulating 10,000 datasets to compare two vaccines.

For a study comparing two vaccines with a sample size of 2,500 per group and with 90% achieving the MPNAT (the chosen measure of vaccine efficacy), it will be possible with 90% certainty to achieve equivalence, with an equivalence margin of δ= +/-2.79%. Similarly, for a vaccine where only 70% achieve the MPNAT, the corresponding minimum equivalence margin that could be attained is δ= +/-4.26%. Thus, from table 1 with a sample size of 2,500 per group our simulations show that with an equivalence margin set at δ=+/-5% we will have sufficient power to ascertain equivalence for all titer levels with ≥90% certainty [2].

At smaller sample sizes the minimum δ that can be evaluated to assess equivalence with 90% certainty is larger. For example, when only 500 participants are included per group, the minimum equivalence margins are δ =+/-8.87% and δ=+/-13.54% for vaccines where 90% and 70%, respectively, achieve the MPNAT.

The sample size computations outlined in table 1 are based on comparing two groups. The study will include four groups leading to six unique comparisons, and the final analysis should therefore allow for multiple testing. Further, such comparisons are not independent of each other and we will investigate how to allow for this so as to best preserve the precision of the equivalence trial. This will be a natural extension of the current simulation-based approach.

Table 1 The minimum equivalence margin (δ) that can be attained with 90% probability when comparing two vaccine groups, for each given sample size and percentage attaining the minimum protective titer level (MPNAT)

		Minimum equivalence margin (δ)					
Total sample size (N)	Sample size per group (n)	Percentage attaining MPNAT (%)					
		40%	50%	60%	70%	80%	90%
500	250	14.41	14.91	14.41	13.54	11.75	8.87
1,000	500	10.12	10.39	10.23	9.57	8.35	6.26
2,000	1,000	7.19	7.37	7.21	6.77	5.87	4.41
5,000	2,500	4.55	4.65	4.56	4.26	3.74	2.79

11.3 Analysis of safety parameters

A quantitative description of serious and non-serious adverse events will be presented.
A qualitative presentation of SAEs will be provided.

11.4 Interim analysis

No formal interim analysis is planned in the sense that a rejection of equivalence will not terminate the study. Instead, monthly reports will evaluate the titer levels to generate signals of insufficient vaccine protection, such that participants can be considered for renewal of their vaccination.

12. End of trial

Within 90 days after the trial completion the Sponsor must inform the Danish Medicines Agency and Ethics Committee about the completion. The result of the trial must be submitted within 12 months.

12.1 Early termination of the trial

The Sponsor reserves the right to terminate the trial under the following conditions:

- Safety concerns
- Proven lack of efficiency
- If an interim statistical analysis shows that the trial has no scientific value or too low power

If the trial is prematurely terminated or suspended, the investigator should promptly inform the subjects and ensure appropriate therapy and follow-up. Furthermore, the investigator and/or Sponsor should promptly inform the pertinent ethics committee and regulatory authorities.

12.2 Subject Discontinuation

The subject will be advised in the informed consent form that he/she has the right to withdraw from the trial at any time without prejudice. Where discontinuation from the trial is initiated by the subject, the investigator is to ascertain the primary reason for discontinuation from the list below:

- Subjects decision to discontinue
- An AE for which the investigator did not consider discontinuation from the trial necessary
- Co-existing disease
- Withdrawal of consent
- Other reasons

The subject may at any time be discontinued from the trial at the discretion of the investigator

Subjects must be discontinued from the trial under the following circumstances:

- If, in the investigator's opinion, continuation in the trial would be detrimental to the subject's well-being
- If informed consent is withdrawn

In all cases, the primary reason for discontinuation must be recorded in the CRF and in the subject's medical records. Follow-up on the subject is necessary to establish whether the reason was an AE. If so, this must be reported in accordance with the appropriate procedures.

Data obtained until discontinuation will be entered in the clinical database and used for statistical analyses.

In cases where a subject withdraw consent, data and samples collected up until the withdrawal will be erased/discharged as far as at all possible (which means if not already included in finalised statistical analysis) and not in the future included in any analysis of the study.

13. Administrative procedures

13.1 Sponsor & Data and Statistical Centre

Study Sponsor and Data and Statistical Centre is Centre for Health and Infectious Diseases Research (CHIP) Department of Infectious Diseases, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark, under the direction of Professor Jens Lundgren.

The DSC will handle data and sample management, bioinformatic processing, research coordination. Including securing the infrastructure to maintain high validity of data and sample collection, storage, statistical analysis, bioinformatic processing and coordination between the involved academic and government partners.

DSC provides the Danish Medicines Agency (the principle receiving entity) with regular monthly reports in an agreed format. The reports will consist of two principle sections: one on operational implementation and data quality, and one on outcomes. Additional analyses may prompt the review of these reports. These reports are considered confidential except otherwise agreed on by the respective steering committee

13.2 Principal Investigator & Co-ordinating Centre

Study Principal Investigator is Professor, MD, Ph.d., DMSc. Lars Østergaard, head of the coordinating center Department Infectious Diseases, Aarhus University and Aarhus University Hospital.

13.3 Governance

The study is overseen by a consortium, within which two steering committees (one on science and one on operations) are formed, with representation of key stakeholders pertaining to the focus of the respective committee. Each committee has a chair and a co-chair.

For data policy see appendix 4. .

13.4 Infrastructure

One person from each of the five regions (see list of the regional coordinator in annex 2) identifies, interacts with staff at the facilities within the region that is participating in the study, and engages in training and re-training of staff with the aim of maintaining high data quality and follow-up of participants, and ensure that the biobanking of samples functions. Across the five regions, it is anticipated that a total of 20-50 vaccination facilities will engage in the study

The central database allows decentralized secure engagement by multiple persons in the analytic work on the data within the central database, for projects and designated person approved by the scientific steering committee.

13.5 Source data and subject data protection

Prior to start of recording of data from subjects, the investigator will prepare a Source Data Location Agreement to document where the first recording of data is done.

The original signed Informed Consent is defined as source data:

The following data must be source data verifiable in the subject hospital record:

- Subject's date of birth
- Vaccine Batch number (only applicable for participants vaccinated under "Tilvalgsordningen" [J&J vaccine])
- Confirmation of participation in the trial
- Confirmation of subject eligibility (in/exclusion criteria)
- SAEs
  Details must include all relevant information given by the subject of the individual event to complete the e-CRF as required

- SAR/SUSAR in case this should occur
- Subject discontinuation from the trial including reason

The site may choose instead of the Subject record to use the Subject Source Data Document or copies of the Work Sheets as source for the following data:

- Date of each trial visit and telephone contacts (telephone only if study related)
- AEs
  Details in the e-CRF must include all relevant information given by the subject of the individual event to complete the e-CRF as required

The following items may be direct entries in the e-CRF:

- AEs
  Details in the e-CRF must include all relevant information given by the subject of the individual event
- Concomitant diseases and medication
- Relevant medical history

A common e-CRF will be constructed and provide the basis for a central database. Data will in the central database be stored in coded form according to the rules of the Danish Data Protection Agency (Datatilsynet) with whom the trial is registered.

In accordance with the Danish Data Protection Agency data processing will be completed. Afterwards data in paper form will be destroyed and electronic data will be transferred and stored at the Danish Data Archives (Statens Arkiver).

13.6 Biobank

Research Biobank:
Samples will be transported directly to Den Nationale Biobank (DNB) at SSI for processing. The shipment can go together with regular samples collected and transported daily from KMA to DNB and SSI.
Samples will be transported from SSI to Department of Infectious Diseases at AUH for processing.

Biobank for future research:
For participants consenting to this, remaining samples will be stored in DNB for future research.

Sub-study No. 1 and No. 2 Research Biobank
Samples will be transported to Department of Infectious Diseases at AUH for processing.

Sub-study No. 1 and No. 2 Biobank:
After the study, for participants consenting to this, remaining samples will be stored in DNB for future research.

Samples in the Biobank and Sub-Study Biobank will be stored for 25 years provided participant acceptance; hereafter the material will be destroyed. Additional analysis will only be performed after approval by the Committee System on Biomedical Research Ethics.

13.7 Monitoring

Regular monitoring will be performed according to ICH GCP and is the responsibility of Sponsor/CHIP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The monitor will verify that the clinical trial is conducted, and data generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

13.8 Financing

Financial support for the conduct of this study will be given to the Coordinating Center's research account from Danish government who supports the trial with 102 million DKK. Funding will cover coordination of the study,

biobank expenses and payment to participating sites per enrolment of study participants in the study. The principal investigator has no economic relation to the funder.

13.9 Insurance

Patients will be covered according to current regulations by the product-responsibility-insurance for the vaccines and the law on patient-insurance.

13.10 Ethical Considerations

Irrespective of the outcome of the trial it will provide useful information on effectiveness and safety of citizens being vaccinated with one of the SARS-CoV2 vaccines the Danish government has purchased.
The result of the trial will thereby provide physicians and patients with new relevant information guiding their choice of vaccination for SARS-CoV2.

Participant risks and discomfort:
The risks for the participants associated with participation in the trial are considered minimal. Upon enrolment participants have been vaccinated with one of the new vaccines approved for use by the Danish Medicines Agency, and available at the participating sites/units.

The discomfort to the participants includes attending the clinic at the 6 study visits and having whole blood collected at these visits

For participants willing to participate in the sub-study additional samples will be collected. Participation in the sub-study is optional. The participant's right to refrain from the sub-study will be respected, and they will be asked to participate in the main protocol only.

After the participants' participation in the trial, an individual plan for re-vaccination, if required, will be made with the patient according to local guidelines.

14. Publication

The consortium's scientific steering committee decides on the approach to reporting of data into scientific journals. All authors need to fulfil the criteria stated in the Vancouver guidelines.

The Danish Government will not be involved in the publication of the trial results, but will be given time to read the manuscript before submission

The trial is investigator-initiated, and data are owned by the Sponsor. Both positive, negative and in-conclusive study results will be published in and international peer-reviewed scientific journal by the investigators of the study group.

The results of the study will also be reported in EudraCT/clinicaltrialsregister.eu

15. References

1. Robinson, L., & Jewell, N. (1991). Some Surprising Results about Covariate Adjustment in Logistic Regression Models. International Statistical Review / Revue Internationale De Statistique, 59(2), 227-240. doi:10.2307/1403444
2. Flight, L., and Julious, S. A. (2016) Practical guide to sample size calculations: non-inferiority and equivalence trials. *Pharmaceut. Statist.*, 15: 80– 89. doi: 10.1002/pst.1716.

Appendix 1 Investigator Signature

It is hereby confirmed that we at our department will be participating in this project according to the protocol and that we by the sponsor will be funded according to the budget

Investigator _____ Date _____
Signature DD-MMM-YYYY

Department _____
Capital letters or stamp

Address _____

_____

_____

_____

Appendix 2 List of Regional Coordinators

Region	Regional Coordinator
Region Nordjylland	Henrik Nielsen, professor og ledende overlæge, Aalborg Universitetshospital
Region Syddanmark	Isik Johansen, professor og overlæge, Odense Universitetshospital
Region Sjælland	Lothar Wiese, ph.d. og overlæge, Sjællands Universitetshospital Roskilde
Region Midtjylland	Nina Breinholt Stærke, læge og forskningsleder/Lars Østergaard, professor og ledende overlæge, Aarhus Universitetshospital
Region Hovedstaden	Thomas Benfield, professor og overlæge, Hvidovre Hospital

Appendix 3 Sub-study No. 1 (under separate consent)

This appendix provides detailed information pertaining to the sub-study. If not stated otherwise, the text in the master protocol gives the approach that will be taken to establish the National SARS-CoV-2 cohort study.

Background and introduction:

Under separate participant informed consent, a cohort will be established including 250 patients from each vaccine group. Live cells (PBMCs) and PAX tubes (for transcriptomic analysis) will be collected for the participants in this cohort. Several work packages or sub-studies will be embedded within this cohort addressing basic and translational research questions requiring additional sampling of biological material as described below.

Coordination:

The study coordinator (AU) will handle sample management, bioinformatic processing, research coordination in the sub cohort study. Including securing the infrastructure to maintain high validity of data and sample collection, storage, statistical analysis, bioinformatic processing and coordination between the involved academic and government partners. Professional coordination will secure that the four sub-studies (or work packages) will run smoothly and in accordance with the set timelines. The broad groups of partners involve will be kept informed and involved in the project and all the phases and key decisions.

Work package 1 (WP1): Immunogenicity: Biobanking of live cells (liquid nitrogen)

1. Introduction and rationale for the biobanking of live cells

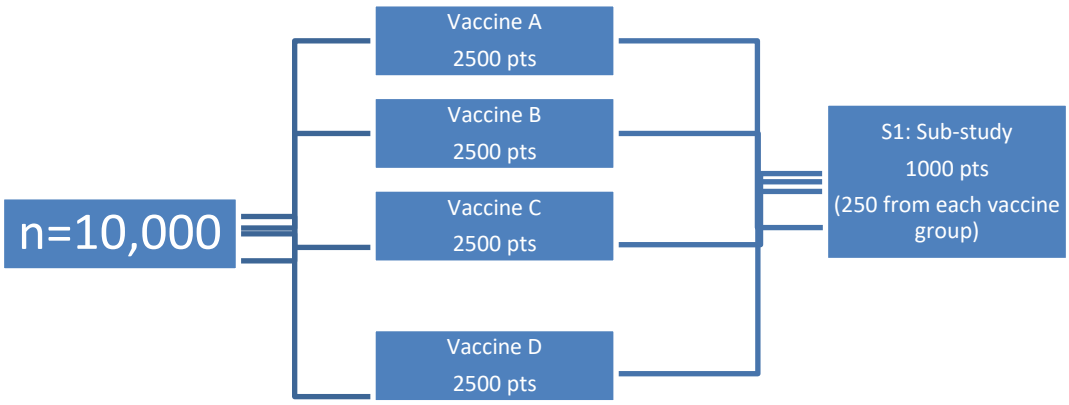
Direct comparison of SARS-CoV-2 vaccines have not been performed. As part of the roll-out of vaccination in Denmark a large biobank of material will be collected to allow assessment of safety and immunogenicity.

2. Aim

To collect live Peripheral Blood Mononuclear Cells (PBMC) from 1000 individuals - 250 individuals from each vaccine group - receiving SARS-CoV-2 vaccination

3. Enrolment

At time of first vaccination, all participants enrolled in the ENFORCE national cohort study are also offered entry into this sub-study under separate consent. For consenting individuals, cell pellets will be stored and used later to extract DNA.



4. Sample collection

For this sub study live cells will be stored in liquid nitrogen to assess cellular immunity.
PBMC will be collected from 3 x 8 mL EDTA-tubes using the MultiMACS automated setup. Following purification cells will be counted and frozen in Liquid Nitrogen in 10 million PBMC aliquots.

5. Data analysis

On 250 individuals from each vaccine group, PBMCs will be collected and stored. Totaling, 6000 PBMC samplings. These specimens will allow for comprehensive immunological comparison to investigate efficacy and safety.

6. **Expected outcomes**

This sub-study aims to generate a well curated and detailed biobank of material to investigate the immunology and safety of the vaccine roll-out.

Work package 2 (WP2): Characterization of polyclonal antibody responses to SARS-CoV-2 variants

1. Introduction and rationale for the antibody responses to characterization

The impact of SARS-CoV-2 genetic variation on vaccine induced immune responses are unknown. In vitro selection studies have highlighted mutations in the Receptor Binding Domain as potential causing monoclonal antibody resistance. The recent reports of the mink derived SARS-CoV-2 variants (Cluster 5) as less sensitive to COVID-19 patient convalescent plasma neutralization further highlight the importance of verifying vaccine-induced antibody breath.

2. Hypothesis

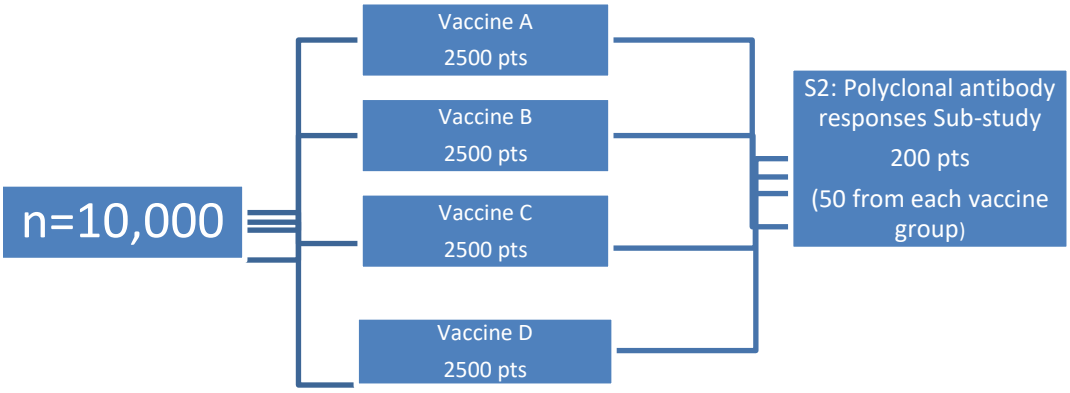
We hypothesize that vaccine-induced polyclonal antibody responses will be able to neutralize SARS-CoV-2 Spike single point mutation variants as well as mink-derived variants.

3. Aim

To perform a comprehensive neutralization profile of serum from vaccinated individuals against a diverse array of SARS-CoV-2 spike genetic variants.

4. Methods

Using samples from the National SARS-CoV-2 cohort study, we will immediately (upon completion of month 3 visit) investigate the ability of SARS-CoV-2 vaccines to generate a polyclonal antibody response that can potently neutralize diverse genetic variants. For this purpose, we will utilise a Vesicular Stomatitis Virus (VSV) pseudotype virus assay. We have generated genetic variants N450D, Y453F, E484K, F490L and the Cluster5 variants (H69+V70 deletion and Y453F, D614G, I692V, M1229I) in the pseudovirus system (all numberings relative to the Wuhan strain https://www.ncbi.nlm.nih.gov/nuccore/MN908947). We will assay plasma antibody neutralization titer in 50 vaccinated individuals (for each SARS-CoV-2 vaccine) and compare neutralization results to those obtained using convalescent plasma from 50 COVID-19 recovered patients. Further we will perform ACE2 blocking experiments on the same cohort using the mesoscale plate format. This will allow for rapid confirmation that vaccine induced polyclonal antibody responses can effectively neutralize diverse SARS-CoV-2 genetic variants as well as mink-derived variants.



6. Data analysis and power calculations

No pre-existing data exists on SARS-CoV-2 vaccine induced antibody coverage, but with an expected geometric mean titer of neutralizing antibodies of 500 and SD of 125. Evaluating 50 individuals receiving the four separate vaccines assuming and alpha of 0.05 and a power of 90 we will be able to detect a decrease in neutralization titer to 420 (to any of the genetic variants).

7.Expected outcomes

This will allow for rapid confirmation that vaccine induced polyclonal antibody responses can effectively neutralize diverse SARS-CoV-2 genetic variants as well as mink-derived variants and will further allow us to bench-mark the results to a standardized high-throughput plate format

Work package 3 (WP3): Characterization of adaptive immune response in individuals with breakthrough infections

1. Scientific rationale:

No clear immune correlate of protection against SARS-CoV-2 infections exist in humans. Levels of neutralizing antibodies have been shown to protect both SARS-CoV-2 challenged hamsters and monkeys. However, clear evidence of significant cellular T cell immune responses has been shown following both vaccination and natural infections.

Poor immune responses to SARS-CoV-2 vaccination will likely increase an individual's risk of breakthrough infection. Several characteristics have generally been associated with vaccine hypo-responsiveness such as age >65 years, smoking, comorbidities, immunosuppression, cancer, and immune-deficiency. However, some individuals who have no known risk factors also respond poorly to vaccines. By combining the detailed characterisation of SARS-CoV-2 vaccine-responses with comprehensive profiling of soluble plasma markers (e.g. using plasma proteomics), it may be possible isolate biomarkers for vaccine-responsiveness that can be used to identify individuals at high-risk of vaccine failure.

2.Hypothesis

We hypothesize that individuals experiencing breakthrough infections following vaccination have aberrant immune responses

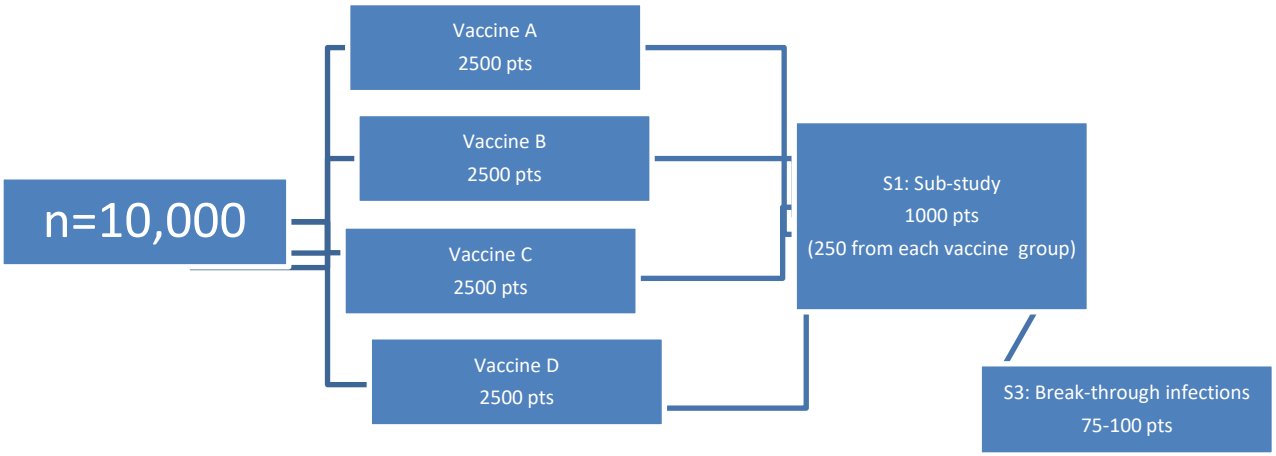
3.Aim

To perform a detailed T cell immune memory characterization and an in-depth serological profile of antibody levels to epitopes on the SARS-CoV-2 Spike protein

4.Methods

Individuals that experience breakthrough infections following vaccination will be matched 1:2 to vaccines not testing positive for SARS-CoV-2. Only individuals that are part of the sub-study cohort with longitudinal isolated PBMCs will be included. We aim to perform the analysis on 75-100 individuals with breakthrough infections. Serological profiles of SARS-CoV-2 Spike epitopes will be performed at visit 2 and up to the visit prior to positive diagnosis. We will utilize the information from the core antibody analysis on all study participants based on the Mesoscale multiantigen serological test including both the N-terminal Domain (NTD), The Receptor Binding Domain (RBD), the complete Spike (S) protein and the Nucleocapsid (NC) protein as antigens.

The remaining workflow is similar to sub-study 2 (S2) on the Characterization of polyclonal antibody responses to SARS-CoV-2 variants. The Mesoscale ACE2 competition assay will be used to score the receptor blocking potential of antibodies raised by vaccination. Further, we will stimulate PBMCs from visit 2 and up to the visit prior to positive diagnosis with overlapping peptides of the Spike protein and investigate bot CD4 and CD8 T cells responses using the Activation Induced Marker (AIM) assay. This will allow for sensitive and quantitative detailed T cell memory phenotype responses.



5.Data analysis and power calculations:
No pre-existing data exists on immune correlate of protection in humans.

6. Expected outcomes:
The results are expected to highlight which immune parameter that is essential for SARS-CoV-2 protection. This will be crucial knowledge to ascertain the level of immunity required to maintain protection and to enable identification of individuals in need of other prophylactic measures.

Work package 4 (WP4): Cellular Immunity: Longitudinal immunoprofiling of vaccine responses in SARS CoV-2 vaccinated individuals

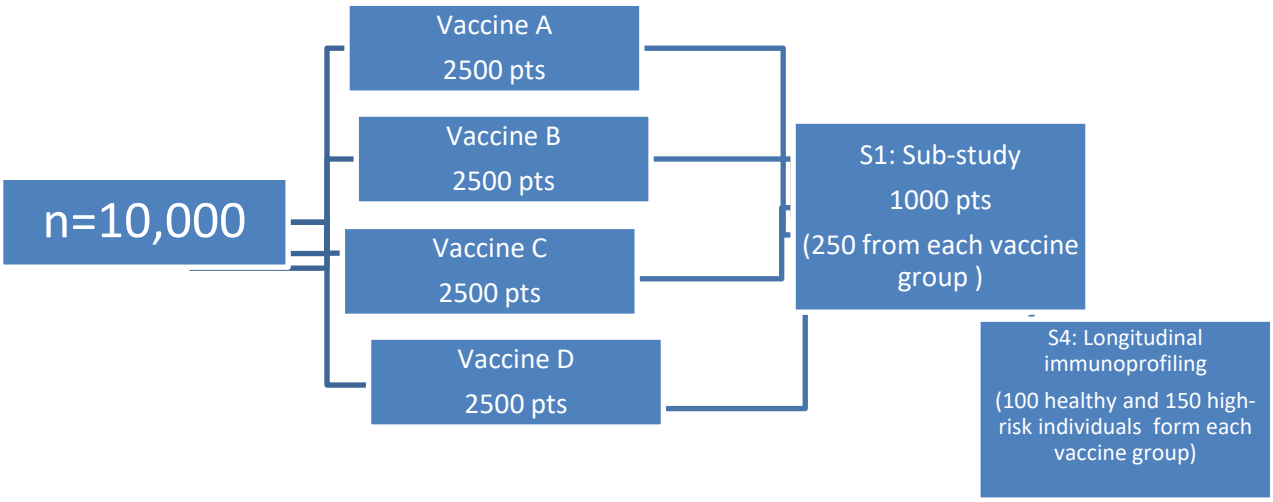
1.Scientific rationale:
Neutralizing antibodies have been shown to protect against SARS-CoV-2 but T cell immune responses also seem to play an important role in protection against COVID-19. However, a multi-vaccine comparison focusing on the magnitude and duration of protection against COVID-19 following SARS-CoV-2 immunization is unlikely to become available from the ongoing phase 3 trials. In addition, phase 1 and 2 trial data on vaccine responses following immunization with different types of SARS-CoV-2 vaccines are difficult to compare due to lack of standardized assays and sample collection time points.

CD4+ and CD8+ T lymphocytes are the primary source of adaptive cellular immunity against coronavirus infections. Cryopreserved live cells are needed for characterization of T cell immunity. Thus, for 250 individuals from each vaccine group, we will conduct a detailed in-depth comparison of vaccine-induced T cell immunity. We aim to enrol a high proportion of COVID-19 high-risk individuals because this group of individuals often also display reduced immune responses to vaccination. Briefly, peripheral blood nuclear cells (PBMCs) from baseline and up to the final study visit at month 24 will be stimulated with overlapping peptides of the Spike protein. Flow cytometry is then applied to investigate both CD4+ and CD8+ T cells responses using the Activation Induced Marker (AIM) assay. This will allow for sensitive and quantitative detailed T cell memory phenotype responses to SARS-CoV-2.

2.Hypothesis:
We hypothesize that the emerging SARS-CoV-2 vaccines differ significantly in terms of their ability to induce cellular immunity and as well as in their neutralizing antibody profile.

3.Aim:
To perform a longitudinal in-depth serological profiling of antibodies to epitopes on the SARS-CoV-2 Spike protein and a detailed T cell immune memory characterization for each approved SARS-CoV-2 vaccine.

4.Methods:
Individuals that are part of the sub-study cohort with longitudinal isolated PBMCs will be included in more comprehensive immunoprofiling. Broader serological profiles of SARS-CoV-2 Spike epitopes will be performed at each visit until month 24. The Mesoscale ACE2 competition assay will be used to score the receptor blocking potential of antibodies raised by vaccination. Further, we will stimulate PBMCs from baseline and up to the 24 months visit with overlapping peptides of the Spike protein. Flow cytometry will be used to quantify both CD4+ and CD8+ T cells responses using the Activation Induced Marker (AIM) assay. This will allow for sensitive and quantitative detailed T cell memory phenotype responses. We aim to perform this embedded comprehensive profiling analysis on approx. 100 healthy and 150 high-risk individuals immunized with each approved SARS-CoV-2 vaccine.



5.Data analysis and power calculations:
T-lymphocyte assays for longitudinal in-depth profiling and quantification of SARS specific CD4+ and CD8+ T lymphocytes for at least 1200 individuals at 6 different time points.

We will compare the longitudinal antibody and cellular SARS-CoV-2 immune responses between the different vaccines that are introduced in Denmark. We will also compare vaccine responses between healthy and high-risk individuals to identify potential protection issues for each vaccine Finally, we will use the immunological data from the WP on breakthrough infections to infer protective levels of antibodies and/or cellular immunity for each vaccine.

The multiantigen serology, Activation Induced Marker (AIM) assay, ACE2 blocking and T cell immunological analyses will be centralized at the core immuno-virological lab at Aarhus University Hospital.

6.Expected outcomes:
This work package aims to apply a stringent, comprehensive and uniform assessment of the serological and cellular immunity for each new SARS-CoV-2 vaccine. This will allow for a detailed cross-vaccine comparison and address potential shortcoming for individual vaccines with special emphasis on the durability of SARS-CoV-2 protection among high-risk individuals.

Appendix 4 Data Policy/Research Proposals to ENFORCE

Who can submit a research proposal to the ENFORCE Consortium?

Proposals to study new issues in the ENFORCE Consortium can be made by study investigators and by external researchers.

How is a proposal evaluated?

All proposals are reviewed by the ENFORCE Steering Committee.

Initially, the Steering Committee evaluates the proposal for:

- Scientific relevance and importance
- Priority compared to other ongoing projects
- Feasibility
- Resources
- Likely success of proposal (incorporating concerns about power)
- Assessment of whether the aims of the proposal are within the stated aims of ENFORCE

If the content of the proposal is within the stipulated agreement (as specified in the study protocol incl. appendixes), the ENFORCE SC may autonomously decide whether the proposal should be approved or not. In general, proposals that fall outside the stated aims of the ENFORCE study will not be considered further.

What should be taken into consideration in the proposal?

- Scientific rationale
- Draft analyses plan
- Sample size considerations
- Use of data that are available as part of the ENFORCE database
- Financial costs
- Additional labour
- Timelines

How long time does it take to get a reply?
Based on the quality of the proposal, the schedule of meetings of the ENFORCE Steering Committee, the usual maximum time to obtain a reply is 2 months.

Who can analyse the ENFORCE dataset?
The complete ENFORCE database is kept at the coordinating centre in Copenhagen. Only personnel authorized access to the database from the coordinating centre can obtain admittance to analyze the dataset.

Appendix 5 Information of the vaccines

5.1 COMIRNATY - BioNTech Manufacturing GmbH

Comirnaty is a vaccine for preventing coronavirus disease 2019 (COVID-19) in people aged 16 years and older. Comirnaty contains a molecule called messenger RNA (mRNA) with instructions for producing a protein from SARS-CoV-2, the virus that causes COVID-19. Comirnaty does not contain the virus itself and cannot cause COVID-19.

The active substance is a single-stranded, 5´-capped messenger RNA produced using a ell-free in vitro transcription from corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2

A very large clinical trial showed that Comirnaty was effective at preventing COVID-19 in people from 16 years of age.

The trial involved around 44,000 people in total. Half received the vaccine and half were given a dummy injection. People did not know whether they received the vaccine or the dummy injection.
Efficacy was calculated in over 36,000 people from 16 years of age (including people over 75 years of age) who had no sign of previous infection. The study showed a 95% reduction in the number of symptomatic COVID-19 cases in the people who received the vaccine (8 cases out of 18,198 got COVID-19 symptoms) compared with people who received a dummy injection (162 cases out of 18,325 got COVID-19 symptoms). This means that the vaccine demonstrated a 95% efficacy in the trial.

The trial also showed around 95% efficacy in the participants at risk of severe COVID-19, including those with asthma, chronic lung disease, diabetes, high blood pressure or a body mass index ≥ 30 kg/m2.

The vaccine is used according to the approved Summary of Product Characteristics

5.2 COVID-19 Vaccine Moderna dispersion for injection - MODERNA BIOTECH SPAIN S.L.

COVID-19 Vaccine Moderna is a vaccine used to prevent COVID-19 caused by SARS-CoV-2. It is given to adults aged 18 years and older.

The active substance in COVID-19 Vaccine Moderna is mRNA encoding the SARS-CoV-2 Spike protein. The mRNA is embedded in SM-102 lipid nanoparticles.

As COVID-19 Vaccine Moderna does not contain the virus, it cannot cause COVID-19.

COVID-19 Vaccine Moderna stimulates the body’s natural defenses (immune system). The vaccine works by causing the body to produce protection (antibodies) against the virus that causes COVID-19. COVID-19 Vaccine Moderna uses a substance called messenger ribonucleic acid (mRNA) to carry instructions that cells in the body can use to make the spike protein that is also on the virus. The cells then make antibodies against the spike protein to help fight off the virus. This will help to protect against COVID-19.

The vaccine is used according to the approved Summary of Product Characteristics

5.3 COVID-19 Vaccine AstraZeneca suspension for injection - AstraZeneca AB, Södertälje, Sweden

COVID-19 Vaccine AstraZeneca is indicated for active immunisation to prevent COVID-19 caused by SARS-CoV-2, in individuals 18 years and older.

One dose (0.5 ml.) contains Chimpanzee Adenovirus encoding the SARS-CoV-2 Spike glycoprotein (ChAdOx1-s), not less than 2.5 x 10^8 infectious units (Inf.U)

COVID-19 Vaccine AstraZeneca is a monovalent vaccine composed of a single recombinant, replication-deficient chimpanzee adenovirus (ChAdOx1) vector encoding the S glycoprotein of SARS-CoV-2.
The SARS-CoV-2 S immunogen in the vaccine is expressed in the trimeric pre-fusion conformation, the coding sequence has not been modified in order to stabilise the expressed s-protein in the pre-fusion conformation.

Following administration, the S glycoprotein of SARS-CoV-12 is expressed locally stimulating neutralising antibody and cellular immune responses. which may contribute to protection to COVID-19.

The vaccine is used according to the approved Summary of Product Characteristics

5.4 COVID-19 Vaccine Janssen suspension for injection/Covid-19 vaccine (Ad26.COV2-S [recombinant])

COVID-19 Vaccine Janssen is indicated for active immunisation to prevent COVID-19 caused by SARS-CoV-2, in individuals 18 years and older.

One dose (0.5 ml.) contains Adenovirus type 26 encoding the SARS-CoV-2 spike glycoprotein* (Ad26.COV2-S), not less than 8.92 $\log_{10}$ infectious unites (Inf.U).
*Produced in the PER.C6 TetR Cell Line and by recombinant DNA technology.
The product contains genetically modified organisms (GMOs)

COVID-19 Vaccine Janssen is a monovalent vaccine composed of a recombinant, replication-incompetent human adenovirus type 26 vector that encodes a SARS-CoV-2 full-length spike (S) glycoprotein in a stabilised conformation. Following administration, the S glycoprotein of SARS-CoV-2 is transiently expressed, stimulating both neutralising and other functional S-specific antibodies, as well as cellular immune responses directed against the S antigen, which may contribute to protection against COVID-19.

The vaccine is used according to the approved Summary of Product Characteristics

Appendix 6 ENFORCE Sub-study 2 (under separate consent)

This appendix provides detailed information pertaining to the sub-study 2 to the ENFORCE master protocol. If not stated otherwise, the text in the master protocol gives the approach that will be taken to establish the National SARS-CoV-2 cohort study.

1. Background and introduction

Vaccination may elicit an immunological reaction which may involve platelet activation and clinical disease. ENFORCE is part of the critical infrastructure to support the Danish vaccination plan. Hence, there may be a need to collect additional information regarding such “issues of concern” as one or several vaccines are being introduced and/or reintroduced.

In order to accommodate a more careful characterisation this sub-study has been designed.

Under separate participant informed consent, the sub-study will establish a cohort of subjects from the ENFORCE master protocol. The sub-study 2 will be activated upon decision by the ENFORCE Scientific Steering Committee provided that the Danish Health Authority and/or Medicines Agency agrees that an “issue of concern” has arisen for one or more vaccines. An appropriate number of participants in ENFORCE vaccinated with other vaccines without such “issues” of concern” may also be invited to participate in the sub-study as “controls”. There is no predefined number of subjects for the sub-study.

Lab. values

The laboratory assessments made in conjunction with implementing this sub-study will need to be adaptive depending on the issue(s) of concern. The three main domains of sample collection are:

i. biochemistry incl. haematology performed at local routine lab;

Hemostasis (in Danish)

- B – Trombocytter
- B – Hæmoglobin
- B – Leukocytter + type
- P – CRP; massek
- P – Fibrinogen; massek.
- P – D-dimer
- P – Koagulationsfaktor VIII (enz.)
- P – Koagulation, overflade-induceret (APTT)
- P – Koagulation, vaevsfaktor-induceret (KFNT/INR)
- P – Antitrombin; arb.stofk. (enzymatisk metode.; faktor Xa- og/eller IIa-metode)
- P – Protein C (aktiveret) resistens; rel.tid (med/uden faktor V)
- P – Protein C (arb.stofk.)
- P – Protein S (frit); arb.stofk.
- S – Antistoffer mod β2-glycoprotein-I (IgG og IgM)
- P – Cardiolipin-antistof (IgG og IgM)
- P – Lupus antikoagulans
- ADAMTS13 (NPU59213): Blå3,5S og Blå2H
- vWF (NPU28493): Blå3,5S og Blå2H
- TEG/ROTEM samt Multiplate

ii. plasma and serum for additional analyses in batches; live cells (PBMC) for characterisation of cell phenotype and activation status

Biomarkers and serology (in Danish)

- Immunaktivering og inflammation: CRP, TNFa, IL-1, IL-6, IL-10 mv.
- Trombocytaktivering: sCD40L, soluble glycoproteiner, degranuleringsmarkører (PF4, vWF, p-selectin, annexin V (surogat for mikropartikler)
- Endothelaktivering/skade: syndecan-1, thrombomodulin, sE-selectin, ICAM, VCAM, vWF Koagulationsaktivering: Protein C, aktiveret protein C, protein S, antitrombiin, d-dimer mv
- Adenovirus serologi status

iii. PAX tubes (for transcriptomic analysis) PAX tubes for transcriptomic analysis using RNAseq or similar methodology. The purpose of the transcriptomic analysis is to determine the impact of vaccination on epigenetic regulation, up- or downregulation of signalizing pathways (e.g. pro-inflammatory pathways, coagulatory pathways, vascular adhesion pathways), and immunologic phenotyping. No genetic analyses will be performed. Transcriptomic data will not be used for genetic analyses either.

Samples will be collected typically at day 7 after (first and second) vaccination and only once, but maybe needed to be repeated depending on the issue of concern.

2. Scientific rationale

This ENFORCE sub-study will implement up to two extra visits 7 days (+/- 5 days) after the 1st and 2nd SARS-CoV-2 vaccination to be able to address specific issues of concerns. Pre vaccination sampling will occur as part of the ENFORCE master protocol and Sub-study No. 1; except for routine biochemistry performed at local lab, which may be collected in Sub-study No. 2

3. Hypothesis

Relevant samples from the three domains described above under section 1 will be collected in order to characterise the body's reaction to the vaccine. Sampling will enable the analysis of changes in inflammation, coagulation, epigenetic regulation, and cell phenotypes caused by the SARS-CoV-2 vaccine.

4. Aim

With two new visits 1b and 2b 7 days (+/- 5 days) after first and second vaccination date the aim is to collect additional biological samples that can be used to address changes in inflammation, coagulation, epigenetic regulation, and cell phenotypes from before the first SARS-CoV-2 vaccine to 7 days after vaccination. The visit and sample collection maybe repeated as needed at later timepoints depending on the issue of concern.

5. Enrolment

In principle, all ENFORCE participants can be invited to take part in the sub-study 2, but we will specifically try to recruit as many individuals as possible from the ENFORCE sub-study 1. Because for the sub-study 1 subjects, we have already collected blood cells and PAX mRNA gene stabilizing tubes (in addition to serum and plasma) prior to vaccination. Thus, collecting new samples 7 days after vaccination will provide a unique opportunity to compare changes in soluble markers, cell characteristics and activation, and cell transcription from pre- to post-vaccination.

6. Sample collection and analysis

Proposed biochemistry samples for analysis at routine local biochemistry department includes leucocyte count and differential, thrombocyte count, CRP, and possible other biomarkers incl D-Dimer and markers of organ dysfunction. A maximum of 80 ml blood will be drawn at each visit, but no more than 240 ml will be drawn in total.

7. Expected outcomes

The comparison of pre-and postvaccination samples will enable the identification of any changes in soluble markers, cell characteristics and activation, and cell transcription related to each vaccine type.

Appendix 7 Transfer of Participants from ENFORCE PLUS

This appendix will transfer the participants from the ENFORCE PLUS study, who all by now have completed their Visit 3 (3 months after vaccination) and therefore already are in the follow-up phase, to be part of the ENFORCE study and the REDCap Database for this study.

Originally the plan was to enrol around 1.000 persons with the vaccine from Johnson & Johnson/Janssen (J&J) under “Tilvalgsordningen” as a medical prescription. However only 25 participants in total were enrolled in the ENFORCE PLUS study, before “Tilvalgsordningen” was closed, and no further participants were included in the protocol.

The primary objective of the ENFORCE PLUS was to assess if the SARS-CoV-2 adenovirus vector vaccine from J&J results in change in number and activation of platelets and anti-PF4 level. It will also compare whether the J&J vaccine is causing a greater activation of platelets and anti-PF4 than the mRNA vaccines. The Danish Medicines Agency approved the vaccine from J&J for use in Denmark, however it never became part of the national vaccine programme.

As a Sub-study of individuals receiving the Astra-Zeneca (adenovirus vector) vaccine to clarify whether this vaccine induces the production of anti-PF4 antibodies, already is part of the ENFORCE protocol (Sub-study 2/Appendix 6), it is hereby the intention to complete the remaining follow-up visits of the 25 participants from the ENFORCE PLUS study, to the ENFORCE protocol and be able to analytically merge the two adenovirus vector vaccinated groups, which anyway was planned originally between the two protocols at the end of both studies.

All 25 participants have signed a specific informed consent to receive the J&J vaccine as stipulated in the regulations from the Danish Health Authority and entering the ENFORCE PLUS study. The participants also consented to their data at some point would be transferred to the ENFORCE database, there seems not to be any issues to continuing the follow-up visits as part of the ENFORCE protocol. All assessments will be performed as originally described in the ENFORCE PLUS protocol, as the follow-up part of the two protocols are identical.

In addition, it is the same study staff performing all the participant visits at the study sites for both the ENFORCE and ENFORCE PLUS studies. Therefore, it makes a lot of the practicalities easier at the study sites, by handling the participants in the same e-CRF system (database). Based on the individual vaccines in the ENFORCE protocol the 25 ENFORCE PLUS participants will always be held in a separate arm in the database.

When this version of the protocol including Appendix 7, is approved by both the Danish Medicines Agency and the Ethics committee - the ENFORCE PLUS study will be closed.

The 25 participants will be informed of the closure of the ENFORCE PLUS and the possible transfer to ENFORCE, first orally (via telephone contact from the study staff) hereafter the written Subject Information describing the transfer will be forwarded to the individual via e-boks. The individual participant will hereafter have the possibility of contacting the study staff to arrange a site visit for further information, if they want to continue their participation.
The participants must be informed of the transfer at their next planned study visit in ENFORCE PLUS at the latest.