The ENFORCE PLUS trial.

The purpose of ENFORCE PLUS was to investigate the efficacy and safety of the SARS-CoV-2 vaccine from Janssen and evaluate if the vaccine creates an activation of the vaccinated person's platelets so that inappropriate antibodies are formed. It was also the idea to evaluate whether the Janssen vaccine caused a larger activation of the platelets than the vaccines used in the national vaccination programme.
In addition, the antibodies against SARS-CoV-2 over 2 years will be evaluated the efficacy of the vaccine over time compare this with the other SARS-CoV-2 vaccines.

The difference between the ENFORCE trial (EudraCT no.2020-006003) and this ENFORCE PLUS trial was the Janssen vaccine was given by the trial staff and the ENFORCE participants received their vaccination in the Danish Vaccination Programme.

Only 25 out of the 1,000 expected participants were included in the trial before an investigation by The European Medicines Agency (EMA) stopped the use of the vaccine, due to the notifications from the US of a possible connection between the vaccine and cases of the rare disease VITT, with a combination of a combination of blood clots in unusual places, a low platelet count, and multiple episodes of bleeding.

Since all 25 participants had completed their third visit (three month after their vaccination) and already were in the follow-up phase, completely identical to the ENFORCE protocol all the participants were transferred for their follow-up to the ENFORCE database.

The primary objective of the ENFORCE PLUS protocol is to investigate whether the SARS-CoV-2 adenovirus vector vaccine from Janssen resulted in a change in the number and activation of platelets and anti-PF4 antibody levels and whether any activation is increased compared to the mRNA vaccines.

A sub-study was already in the ENFORCE protocol (Sub-study 2/Appendix 6) with participants who have received the vaccine from Astra-Zeneca (also an adenovirus vector vaccine), which investigates the same as the above.

It was already planned to combine the two adenovirus vector vaccinated groups (ENFORCE PLUS and ENFORCE Sub-study 2) for analysis purposes, which is why it made sense to transfer the last visits for the 25 ENFORCE PLUS participants directly to the ENFORCE database when the participants came for the next visits.

When the 25 participants signed their informed consent, they gave permission for their data to be transferred to the ENFORCE database at some point, which is why no problem was seen for the last visits to be entered directly into the ENFORCE database by the trial staff at the individual centres.

All parameters were performed exactly as originally described in the ENFOCE PLUS protocol, as it is completely identical to the ENFORCE protocol, and thus it made no difference to the participants. The same trial staff performed the participant visits in both trials.

The individual "vaccine arms" were kept separate in the ENFORCE protocol, and this will of course also apply to the participants from ENFORCE PLUS.

ENFORCE Plus Participants

An overview of the participants originally enrolled in ENFORCE Plus, and transferred to follow-up in ENFORCE is hereby reported:

Overview

A total of 25 participants who received a first dose of the Janssen vaccine were enrolled in the ENFORCE Plus study during July 2021. Five participants have subsequently withdrawn from the study.

An overview of the study visits is given in Table 1. Twenty-two (88%) participants received a second vaccine dose of either Pfizer-BioNTech or Moderna a median of 134 days after their first dose (IQR 170-66). Less than 5 participants have subsequently received a second dose of the mRNA vaccine.

Table 1 Number and percentage of participants who received the Janssen vaccine completing each study visit

	Janssen/J&J (N=25)
Safety visit 1b (N, %)	23 (92.0)
Time to safety visit 1b (median, IQR)	7 (5.8)
Safety visit 1c (N, %)	23 (92.0)
Time to safety visit 1c (median, IQR)	31 (28, 35)
Visit 3 (3 months after first vaccination) (N, %)	21 (84.0)
Days from first vaccine to third study visit (median, IQR)	92 (90,94)
Visit 4 (6 months after first vaccination) (N, %)	21 (84.0)
Days from first vaccine to fourth study visit (median, IQR)	185 (182, 188)

Primary outcome

Out of the 24 participants with data from the Wantai Total Ig ELISA assay at enrolment <5 participants were positive for antibodies. At visit 3 and 4, all participants with data were positive for antibodies. From the multiantigen serological tests, the geometric mean (GM) and 95% confidence intervals (CI) for the antibody levels against the Receptor Binding Domain, and the complete Spike protein at each study visit are reported in Table 2.

Table 2 Presence of antibodies at study visit, Receptor-Binding Domain (RBD), Spike antibody, among participants who received the Janssen vaccine

	Vaccine type Janssen/J&J (N=25)
AUH antibody data at visit 1 (enrolment) (N, % of total)	23 (92.0)
AUH antibody data at visit 3 (3 months after first vaccination) (N, % of total)	21 (84.0)
AUH antibody data at visit 4 (6 months after first vaccination) (N, % of total)	21 (84.0)
CoV-2 Receptor-Binding Domain (SERO)	
GM at enrolment (95%CI)	230 (96, 552)
GM at visit 3 (95%CI)	14586 (6827, 31161)
GM at visit 4 (95%CI)	298016 (214679, 413704)
CoV-2 Spike antibody (SERO)	
GM at enrolment (95%CI)	337 (128, 888)
GM at visit 3 (95%CI)	32361 (16526, 63368)
GM at visit 4 (95%CI)	398955 (311946, 510233)

Secondary outcome

Nineteen (76%) participants who received the Janssen vaccine have subsequently had a positive PCR test for SARS-CoV-2 a median of 208 days (IQR 194-255) after their first vaccine dose (Table 3).

Table 3 Number of participants who received the Janssen vaccine testing positive for SARS-CoV-2

	Vaccine type Janssen/J&J (N=25)
Ever tested for SARS-CoV-2 reported via KIDS (N, % of total)	24 (96.0)
Number PCR positive for SARS-CoV-2 reported via KIDS (N, % of total)	19 (76.0)
Days from first vaccine dose to SARS-CoV2 positive test (median, IQR)	208 (194,225)

Safety and Monitoring

There have been no serious adverse events or deaths reported among the 25 participants who received the Janssen/J&J vaccine. Fewer than 5 participants have reported a grade 3 serious adverse event.