Sponsor	University of Cologne Represented by: Prof. Dr. med. Oliver Cornely (Principal Coordinating Investigator, PCI) Department for Internal Medicine I University Hospital Cologne Kerpener Strasse 62 50937 Cologne Germany
Principal Coordinating Investigator	See above National representatives are detailed in a separate list for this multinational trial.
Title of the clinical trial	**A Multinational, Phase 2, Randomised, Adaptive Protocol to Evaluate Immunogenicity and Reactogenicity of Different COVID-19 Vaccines Administration in Older Adults (≥75) already Vaccinated Against SARS-CoV-2 (EU-COVAT-1 AGED)**
Indication	COVID-19 vaccination
Phase	Phase II clinical trial
Type of trial, trial design, methodology	**Please note: This trial (*"EU-COVAT-1_AGED")* is a sub-protocol embedded within the *EU-COVAT* master protocol.** **PLEASE NOTE:** **This Statistical Analysis Plan (SAP) refers primarily to Part B of the clinical trial in which new accruals are randomized to a 4th vaccination (second booster) with either BNT162b2 or mRNA-1273.** **Part A of the present trial in which individuals received a 3rd vaccination (first booster) is closed to further recruitment as of January 13, 2022. With the massive roll-out of booster campaigns throughout Europe, Part A was abandoned because of a poor recruitment rate.** **Individuals in Part A are followed-up as specified in protocol version V04_0 and analyzed descriptively as outlined in Appendix A.**

	This is a randomised controlled, adaptive, multicentre Phase II protocol evaluating different booster strategies in **individuals aged 75 years and older** already vaccinated against SARS-CoV-2. Part B of this trial foresees testing of different vaccines as a **4th vaccination dose** (second booster) for comparative assessment of their immunogenicity and safety against SARS-CoV-2 wild-type and variants in the elderly, a usually neglected population. Additional vaccines and extended follow-up visits can be added through amendments of this sub-protocol. As stated in the *EU-COVAT* master protocol, this trial, i.e., the *EU-COVAT-1_AGED study,* implements a specific safety monitoring strategy (see below). **Randomisation in Part B** Subjects who - **prior to study entry** - got a primary vaccination series and 3rd vaccination dose of either • BNT162b2 + BNT162b2 + BNT162b2 or • BNT162b2 + BNT162b2 + mRNA-1273 or • mRNA-1273 + mRNA-1273 + mRNA-1273 or • mRNA-1273 + mRNA-1273 + BNT162b2 or • ChAdOx-1-S + ChAdOx-1-S + BNT162b2 or • ChAdOx-1-S + ChAdOx-1-S + mRNA-1273 or will receive a **4th vaccination dose** with an allocation ratio of 1:1 to either BNT162b2 or mRNA-1273. Accordingly, there are **6 cohorts** (equalling 12 arms). All individuals who were randomized • to BNT162b2 represent **Group 1**, all individuals who were randomized • to mRNA-1273 represent **Group 2**. Cohorts and arms can be withdrawn or added as deemed necessary according to the criteria specified in this protocol. **Blinding** No blinding is foreseen in this trial
Number of subjects	The number needed has been calculated at 550 for **Part B**. **Sample size calculation with multiplicity adjustment within each cohort**

	When the sample size is 250 per randomized group (Group 1, Group 2) in Part B (275 without dropouts, i.e., assuming 8-10 % dropouts), two-sided simultaneous 95% confidence intervals (with Bonferroni adjustment for 2 simultaneous confidence intervals within a cohort) for a proportion using the large sample normal approximation will extend no more than ±7.1% (percentage points) from the observed proportion. E.g., if the observed proportion is 50% (where the confidence interval is widest), the confidence interval ranges from about 42.9% to 57.1%.
Primary trial objective (part B)	• To compare the immune response between treatment arms **after a 4th vaccination dose** against SARS-CoV-2.
Safety objectives	• To assess the safety of a 4th vaccination dose against SARS-CoV-2 in the study population.
Secondary trial objectives	• To compare the **humoral response** against wild-type SARS-CoV-2 between treatment arms **after a 4th vaccination dose** against SARS-CoV-2. • To evaluate descriptively the **humoral response** against SARS-CoV-2 **variants of concern** between treatment arms after a 4th vaccination dose against SARS-CoV-2. • To evaluate descriptively the long-term humoral immune response of 4th vaccination dose against SARS-CoV-2.
Exploratory objectives **(Part B)**	• To investigate the cellular immune response after a 4th vaccination dose, virus neutralizing capacity against newly emerging variants in bio-banked samples and correlates of interest.
Study end points **(Part B)**	**Primary end point:** • Rate of 2-fold antibody titre increase 14 days **after a 4th vaccination dose** measured by quantitative enzyme-linked immunosorbent assay (Anti-RBD-ELISA) against wildtype virus. (Part B) **Safety endpoints:** • Unsolicited AEs until the end of trial. • Solicited AEs for 7 days after a 4th vaccination dose. • Rate of serious adverse events (SAEs) Grade ≥3 according to the National Cancer Institute Common Toxicity Criteria up to three months after a 4th vaccination dose.

	Secondary end points: • Change in neutralizing antibody titre (Virus Neutralisation Assay) against wild-type 14 days after a 4th vaccination dose, to be determined in a subgroup only. • Change in neutralizing antibody titre (Virus Neutralisation Assay) **against variants of concern** 14 days after a 4th vaccination dose, to be determined in a subgroup only. • Antibody titre level **at 12 months** after a 4th vaccination dose measured by a quantitative enzyme-linked immunosorbent assay (anti-RBD-ELISA assay). • Neutralizing antibody titre (Virus Neutralisation Assay) against wild-type SARS-CoV-2 at 12 months after a 4th vaccination dose, to be determined in a subgroup only. • Neutralizing antibody titre (Virus Neutralisation Assay) **against variants of concern** at 12 months after a 4th vaccination dose, to be determined in a subgroup only. **Exploratory endpoints:** • Change in cellular immune response (CD4+ and CD8+ T cell response) measured by qPCR 14 days after 4th booster dose, to be determined in a subgruoup ony. • Neutralizing antibody titre (Virus Neutralisation Assay) against newly emerging variants in bio-banked samples in a subgroup analysis after 4th vaccination dose, to be determined in a subgroup only. • Correlates of humoral immune response, cellular immune response and viral neutralising capacity against SARS-CoV-2 variants of concern (VOCs), to be determined in a subgroup only.

Diagnosis and Principal inclusion and exclusion criteria (Part B)	**Medical condition or disease to be investigated:**
	
	Prevention of COVID-19 infection.
	
	Principal inclusion criteria:
	
	• Subject is ≥75 years old).
	• Prior to study entry the subject was vaccinated with one of the following vaccination regimens (1st + 2nd + 3rd dose):
	
	  ○ BNT162b2 + BNT162b2 + BNT162b2
	  ○ BNT162b2 + BNT162b2 + mRNA-1273

	○ mRNA-1273 + mRNA-1273 + mRNA-1273 ○ mRNA-1273 + mRNA-1273 + BNT162b2 ○ ChAdOx-1-S + ChAdOx-1-S + BNT162b2 ○ ChAdOx-1-S + ChAdOx-1-S + mRNA-1273 • The last dose of the above listed vaccinations must have been administered at least 1 month prior to study entry. Vaccination status should be documented in the source data and will be captured in the eCRF. • Written informed consent from subject has been obtained. **Principal exclusion criteria**: • Prior to study entry the subject got vaccinated with a regimen not included in the list given above. • Last anti-SARS-CoV-2 vaccine dose administered less than one month prior to study entry. • Vaccination against a disease other than COVID-19 within 2 weeks prior to study entry. Only exception: Influenza vaccination which is allowed at any time. • Subjects with any significant or uncontrolled disease posing a risk due to vaccination as judged by the investigator. • Current immunosuppressive therapy, for example continuous glucocorticosteroid treatment equivalent to >10 mg/day prednisolone. • Subject simultaneously participates in another clinical trials or has participated in the past 30 days. • Subjects unable to report solicited adverse events. • Subject with any contraindications to the vaccines in the trial. A list of contraindications as listed in the Summary of medicinal Product Characteristics (SmPC, the Fachinformation in Germany), if appropriate.
Name of investigational medicinal product (IMP)	• BNT162b2 (Comirnaty®) including any VOC-modified vaccine product • mRNA-1273 (Spikevax®) including any VOC-modified vaccine product

Investigational medicinal product – dosage and method of administration

Intervention in Part A: no longer active - for information only

Cohort	Vaccination prior to study entry	Arm	Study intervention: 3rd vaccination dose	Part A with Cohorts 1 to 3 closed to further recruitment as of January 13, 2022
Cohort 1	BNT162b2 + BNT162b2	1	BNT162b2	
		2	mRNA-1273	
Cohort 2	mRNA-1273 + mRNA-1273	3	BNT162b2	
		4	mRNA-1273	
Cohort 3	ChAdOx-1-S + ChAdOx-1-S	5	BNT162b2	
		6	mRNA-1273	
Control	Control arm of the EU-COVAT subprotocol EudraCT no. 2021-004889-35, a separate sub-protocol embedded within the EU-COVAT master protocol, will be used for a descriptive comparison.			

Intervention in Part B: 4th vaccination dose

Cohort	Vaccination prior to study entry	Arm	Study intervention: 4th vaccination dose*
Cohort 4	BNT162b2 + BNT162b2 + BNT162b2	7	BNT162b2
		8	mRNA-1273
Cohort 5	BNT162b2 + BNT162b2 + mRNA-1273	9	BNT162b2
		10	mRNA-1273
Cohort 6	mRNA-1273 + mRNA-1273 + mRNA-1273	11	BNT162b2
		12	mRNA-1273
Cohort 7	mRNA-1273 + mRNA-1273 + BNT162b2	13	BNT162b2
		14	mRNA-1273
Cohort 8	ChAdOx-1-S + ChAdOx-1-S + BNT162b2	15	BNT162b2
		16	mRNA-1273
Cohort 9	ChAdOx-1-S + ChAdOx-1-S + mRNA-1273	17	BNT162b2
		18	mRNA-1273

<table>
<tr><td></td><td>* administered at least 1 month after the 3rd pre-study vaccination.</td></tr>
<tr><td>IMP or therapy used as a comparator – dosage and method of administration</td><td>No vaccination in the control group of the EU-COVAT subprotocol EudraCT no. 2021-004889-35, a separate sub-protocol embedded within the EU-COVAT master protocol (applies to Part A of the trial, in Part B this is an option only).</td></tr>
<tr><td>Duration of treatment
(Part B)</td><td>Treatment consists of a single 4th dose of each vaccine foreseen in this protocol. Follow up of subject included will last for 12 months.</td></tr>
<tr><td>Time plan
(Part A and B)</td><td>
<table>
<tr><td>First patient first visit (FPFV):</td><td>November 2021</td></tr>
<tr><td>Last patient first visit (LPFV):</td><td>September 2022</td></tr>
<tr><td>Last patient last visit (LPLV):</td><td>September 2023</td></tr>
<tr><td>Analysis</td><td>October 2022 (Primary endpoint analysis)</td></tr>
<tr><td>End of study definition</td><td>The end of study will be on the day of database lock.</td></tr>
<tr><td>End of trial</td><td>November 2023</td></tr>
<tr><td>Final study report:</td><td>December 2023</td></tr>
</table>
</td></tr>
<tr><td>Statistician</td><td>Assoc. Prof. Priv.-Doz. Dr. Franz König
Center for Medical Statistics, Informatics and Intelligent Systems
Medical University of Vienna
Spitalgasse 23,
1090 Vienna
Austria</td></tr>
<tr><td>Statistical methods
(Part B)</td><td>Firstly, the rates of 2-fold increase in antibody titre with multiplicity adjusted 95% confidence intervals (adjusted for arms within each cohort) will be reported for both randomized groups 1 and 2 in Part B.

Secondly, Cohorts of study participants are defined by the different pre-vaccination series eligible for part B of this trial. We compare the 4th vaccination doses of BNT162b2 and mRNA-1273 and compute a two-sided confidence interval. Based on these we test for equivalence of one of the 4th vaccination doses (based on the equivalence margin as outlined in the Reflection Paper of EMA).</td></tr>
</table>

	Thirdly, we compare groups 1 and 2 based on homologous boosting (pre-study 3rd vaccine and on-study 4th vaccine **identicial**) vs. heterologous boosting (pre-study 3rd vaccine and on-study 4th vaccine **different**). Further exploratory analyses will be performed based on different pre-vaccination series. **The primary endpoint analysis** in part B will be performed as soon as single cohorts have completed day 14 of the visit schedule. The primary endpoint analysis will be triggered as soon as for all patients the primary endpoint (14 days after the 4th vaccination dose) has been observed. **Safety analysis.** All clinical safety data will be listed by participant, time from vaccination dose and treatment arm. Continuous variables will be summarised using sample size (N), mean, standard deviation, median, minimum, and maximum. Frequency counts will be reported for categorical data. A Data and Safety Monitoring Committee (DSMC) will review data for decisions on arms incorporation and withdrawal and will ensure the appropriate oversight and monitoring in conducting the clinical trial. **Interim analysis** An interim analysis will be performed as soon as 50% of participants have been recruited within Part B.
GCP compliance	The present trial will be conducted in accordance with the valid versions of the trial protocol and the internationally recognised Good Clinical Practice Guidelines (ICH-GCP), including archiving of essential documents.
Financing	European Commission

1 Objectives of the clinical trial

Primary objective (Part B)

- To compare the immune response between treatment arms after a 4th vaccination dose against SARS-CoV-2.

Safety objective (Part B)

- To assess the safety of a 4th vaccination dose against SARS-CoV-2 in the study population.

Secondary objectives (Part B)

- To compare the humoral response against wild-type SARS-CoV-2 between treatment arms after a 4th vaccination dose against SARS-CoV-2.
- To evaluate descriptively the humoral response against SARS-CoV-2 variants of concern between treatment arms after a 4th vaccination dose against SARS-CoV-2.
- To evaluate descriptively the long-term humoral immune response of a 4th vaccination dose against SARS-CoV-2.

Exploratory objectives (Part B)

- To investigate the cellular immune response after a 4th vaccination dose, virus neutralizing capacity against newly emerging variants in bio-banked samples and correlates of interest.

2 General aspects of trial design

According to section 4.1 of the accompanying master protocol:

This is a randomised, adaptive, multicentre, Phase II master protocol evaluating different booster strategies in individuals against SARS-CoV-2. This protocol allows testing of different booster strategies to assess their immunogenicity and safety against SARS-CoV-2 and its variants. Different trial populations, different vaccines, additional doses and vaccination time points, and extended follow-up visits can be added throughout amendments of the master protocol. The master protocol contains a common control group that does not receive a 3rd nor a 4th vaccination, i.e., no booster dose at all. This control group is used for exploratory comparison as applicable. The trial implements a specific safety monitoring strategy for this control group.

2.1 Specific to this trial:

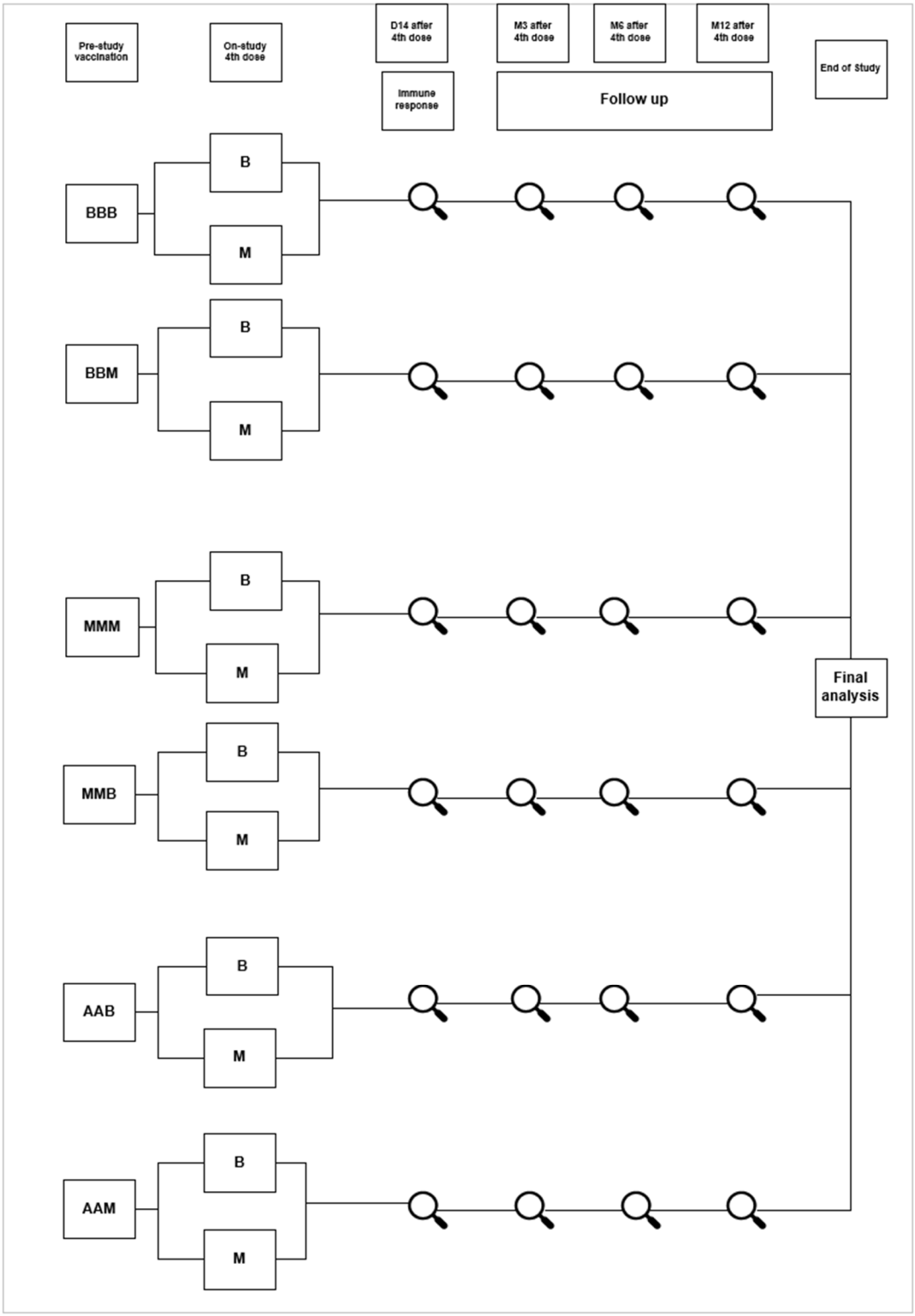
This is a randomised controlled, adaptive, multicentre Phase II protocol evaluating the immunogenicity and reactogenicity of different strategies of a 3rd (Part A) and 4th (Part B) vaccination dose ("boosters") in individuals ≥75 years after a primary vaccination series against SARS-CoV-2. This trial foresees testing of different vaccines as a 3rd or 4th vaccination dose (also referred to as booster vaccinations) for comparative assessment of their immunogenicity and safety against SARS-CoV-2 wild-type and variants in the elderly, a usually neglected population. Additional vaccines and extended follow-up visits can be added by means of amendments to this sub-protocol. As stated in the master protocol, this trial implements a specific safety monitoring strategy for the control group (see below).

Subjects in each cohort will be randomised to one of the arms planned in an equal allocation ratio. In each cohort, the number of arms foreseen at this moment is 2; therefore, the allocation ratio within in each cohort will be 1:1.

Arms can be withdrawn or added as deemed necessary according to the criteria specified in this protocol.

Figure 1 shows a schematic flow chart of the trial design of part B.

Figure 1: Flow chart of the trial design



A: AstraZeneca vaccine ChAdOx-1-S **B:** BioNTech vaccine Comirnaty® **M:** Moderna vaccine Spikevax®
D14: day 14
M3/6/12: month 3/6/12

2.2 Time schedule

The time schedule of this trial is detailed in table 1.

Table 1: Time schedule of the trial (Part A and Part B)

First patient first visit (FPFV):	November 2021
Last patient first visit (LPFV):	September 2022
Last patient last visit (LPLV):	September 2023
Analysis	October 2022 (Primary endpoint analysis)
End of study definition	The end of study will be on the day of database lock.
End of trial	November 2023
Final study report:	December 2023

End of study will be on the day of database lock, as planned 30 days after the last patient last visit.

3 Discussion of trial design

According to section 4.2 of the accompanying master protocol:

Trials under the master protocol allow testing of different booster strategies to assess their immunogenicity and safety against SARS-CoV-2 and its variants. Different trial populations, different vaccines, additional doses and vaccination time points, and extended follow-up visits can be added throughout amendments of the master protocol or trials under the master protocol.

Specific to this trial:

This trial is a sub-protocol embedded within the master protocol EU-COVAT: A Randomised, Controlled, Adaptive, Multicentre, Phase II Master Protocol Evaluating Different Booster Strategies in Individuals Already Vaccinated Against SARS-CoV-2.

This is a randomised controlled, adaptive, evaluator-blinded multicentre Phase II protocol evaluating immunogenicity and reactogenicity of different booster strategies in individuals 75 years and older after a primary vaccination series against SARS-CoV-2. This trial foresees testing of different vaccines as a 3rd (Part A) or 4th (Part B) vaccination dose (refer to section 4.2 in the current protocol) for comparative assessment of their immunogenicity and safety against SARS-CoV-2 wild-type and

variants in the elderly, a usually neglected population. Different vaccines and extended follow-up visits can be added by means of amendments to this trial. As stated in the master protocol, this trial implements a specific safety monitoring strategy for a control group as implemented in the EU-COVAT subprotocol with the EudraCT no.: 2021-004889-35, a separate sub-protocol embedded within the EU-COVAT master protocol (see below). This control group is used for exploratory comparison.

Vaccines used in this trial will be those already marketed and in their commercial presentation. In part B the sample size planned is adequate for a precise enough estimation of the immune response after a 4th vaccination dose.

4 Selection of trial population

According to section 4.4 of the accompanying master protocol:

VACCELERATE has established a European volunteer registry for vaccine trials. A continuously growing number of interested individuals have expressed their interest in this registry. This will allow for fast and efficient recruitment of trial participants.

Subjects to be recruited in the trials under the master protocol will be those already fully vaccinated (all-comers) with no contra-indication against any of the vaccine products in the trial at the moment of inclusion and as specified in respective sub-protocol.

Within each trial, subjects will be stratified (as applicable and specified per sub-protocol) by:

- Gender (female/male).
- Vaccine product used for primary vaccination (BNT162b2; mRNA-1273; ChAdOx-1-S; other)
- Immune status (competent, immunocompromised).

Documented history of prior COVID-19 infection.

Reasons for gender distribution

Subjects included in this trial are expected to be similar to subjects that have been vaccinated within the vaccination programs in each country. Therefore, gender distribution in the present trial is expected to be similar to that of the general vaccinated population and no restrictions will be made with relation to biological sex, unless included in the selection criteria.

Specific to this trial, Part B:

Subjects included in this trial are expected to be similar to subjects that have been vaccinated within the vaccination programs in each country. Gender distribution will be therefore similar to that of the population vaccinated so far. Accordingly, Part B will include 550 subjects that are randomised 1:1 to either BNT162b2 or mRNA-1273. Recruitment of cohorts will be closed when the pre-planned number for each cohort is reached. Stratification is planned according to the pre-study vaccination series eligible for Part B (primary vaccination plus 3rd vaccine dose (= first booster), gender (female/male), documented history of prior COVID-19 infection (yes/no), as applicable and defined also by enrolment criteria.

Inclusion criteria

- Subject is ≥75 years old.
- For study entry in **Part B** the subject was vaccinated with one of the following vaccination regimens (1st + 2nd + 3rd dose):
    - BNT162b2 + BNT162b2 + BNT162b2
    - BNT162b2 + BNT162b2 + mRNA-1273
    - mRNA-1273 + mRNA-1273 + mRNA-1273
    - mRNA-1273 + mRNA-1273 + BNT162b2
    - ChAdOx-1-S + ChAdOx-1-S + BNT162b2
    - ChAdOx-1-S + ChAdOx-1-S + mRNA-1273

- The last dose of the above vaccinations must have been administered at least 1 month prior to study entry. Vaccination status should be documented in the source data and will be captured in the eCRF.
- No contra-indication against any of the vaccine products in the trial.
- Written informed consent from subject has been obtained.

Exclusion criteria

- Prior to study entry the subject got vaccinated with a regimen not included in the above list.
- Last anti-SARS-CoV-2 vaccine dose administered less than one month prior to study entry.
- Vaccination against a disease other than COVID-19 within 2 weeks prior to study entry. Only exception: Influenza vaccination which is allowed at any time.
- Subjects with any significant or uncontrolled disease posing a risk due to vaccination as judged by the investigator.

- Current immunosuppressive therapy, for example continuous glucocorticosteroid treatment equivalent to >10 mg/day prednisolone.
- Subject simultaneously participates in another clinical trials or has participated in the past 30 days.
- Subjects unable to report solicited adverse events.
- Subject participates or participated in Part A of this trial.
- Subject with any contraindications to the vaccines in the trial. A list of contraindications as listed in the Summary of medicinal Product Characteristics (SmPC, the Fachinformation in Germany), if appropriate.
- Use of drugs with significant interaction with the investigational product according to the SmPC or similar documents.
- Diseases or findings that may have a significant effect on the target variables and which may therefore mask or inhibit the therapeutic effect under investigation.
- Subject had COVID-19 or tested positive for SARS-CoV-2 within the last 3 months.
- Persons with any kind of dependency on the principal investigator or employed by the sponsor or principal investigator.
- Legally incapacitated persons.
- Persons held in an institution by legal or official order.

Withdrawal of trial subjects after trial start

According to section 4.5. of the accompanying master protocol:
A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, or compliance/adherence reasons. This is expected to be uncommon. When a participant withdraws before study completion, the reason for withdrawal is to be documented in the eCRF and in the source document. If the reason for withdrawal from the study is withdrawal of consent, then no additional assessments are allowed. The participant will be permanently discontinued from the study intervention and the study at that time. If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the study site records.

5 Treatments to be given

According to section 4.7.1. of the accompanying master protocol:
Vaccines to be evaluated in sub-protocols under the master protocol will be vaccines with marketing authorization at the time of each sub-protocol design and/or vaccines still in clinical development, i.e., pre-authorisation. Vaccines to be administered in the intervention and control arms will be defined in each sub-protocol depending on the hypothesis and the booster strategies tested. Subjects allocated to control arms could receive a 3rd vaccine dose or not receive a 3rd vaccine dose.

Specific to this trial, Part B:
Study vaccines will be given as a 4th dose in subjects already vaccinated with defined prior vaccination strategies (with BNT162b2, mRNA-1271 or ChAdOx-1-S) as per eligibility criteria. The vaccines to be evaluated as 4th vaccination (second booster) dose will be BNT162b2 (Comirnaty®) and mRNA-1273 (Spikevax®). For a detailed overview of all cohorts and arms in this study please refer to Tables 2.1 and 2.2 below.

In Part B there is a minimum interval of 1 month between the on-study 4th vaccination dose and the 3rd pre-study vaccination, with longer intervals as per local regulation or practice.

Table 2.2: **Intervention in Part B - 4th vaccination dose**

Cohort	Vaccination prior to study entry	Arm	Study intervention: 4th vaccination dose*
Cohort 4	BNT162b2 + BNT162b2 + BNT162b2	7	BNT162b2
		8	mRNA-1273
Cohort 5	BNT162b2 + BNT162b2 + mRNA-1273	9	BNT162b2
		10	mRNA-1273
Cohort 6	mRNA-1273 + mRNA-1273 + mRNA-1273	11	BNT162b2
		12	mRNA-1273
Cohort 7	mRNA-1273 + mRNA-1273 + BNT162b2	13	BNT162b2
		14	mRNA-1273
Cohort 8	ChAdOx-1-S + ChAdOx-1-S + BNT162b2	15	BNT162b2
		16	mRNA-1273
Cohort 9	ChAdOx-1-S + ChAdOx-1-S + mRNA-1273	17	BNT162b2
		18	mRNA-1273

** administered at least 1 month after the 3rd pre-study vaccination.*

6 Assignment of trial subjects to treatment groups

According to section 4.7.5 of the accompanying master protocol:

Subject fulfilling selection criteria will be randomised to the different treatment arms in the trial through a central procedure. Randomisation will be stratified according to:

- Vaccine product used for primary vaccination (detailed in the concerned sub-protocol).
- Trial Site.
- Gender (female/male).
- Immune status (competent, immunocompromised as applicable in the concerned sub-protocol).
- Documented history of prior COVID-19 infection (none, asymptomatic, symptomatic/pre-vaccination or post-vaccination).

The participants are randomised into the study arms as defined in each sub-protocol. Randomisation will be implemented by a 24/7-Internet service (ALEA 17.1, FormsVision BV, Abcoude, NL) and prepared centrally by the Institute of Medical Statistics and Computational Biology (IMSB) at the University of Cologne.

Specific to this trial, Part B:

Subjects fulfilling selection criteria will be randomised to 4th dose through a central procedure. Subjects will be randomised in a 1:1 ratio to one of the two treatment groups:

- 4th vaccination with BTN162b2 (Comirnaty®), including modified vaccine product.
- 4th vaccination with mRNA-1273 (Spikevax®), including modified vaccine product.

Randomisation will be stratified as described below:

Stratification is planned according to the pre-study vaccination series eligible for Part B (primary vaccination plus 3rd vaccine dose (= first booster), gender (female/male), documented history of prior COVID-19 infection (yes/no), as applicable and defined also by enrolment criteria.

In this trial, there is <u>no</u> stratification in the randomisation

- according to the status of immune competency of the trial subject.
- according to trial site.

7 Efficacy and safety endpoints

According to section 4.8 of the accompanying master protocol:

Main overarching immunogenicity variables to be included in the sub-protocols are those standard tests showing the immune response to SARS-CoV-2 vaccines, as described below.

Overarching safety variables of interest include unsolicited and solicited adverse events day 14, as applicable, after a 4th vaccination dose administration and severe adverse events for the duration of the trial.

Specific to Part B of this clinical trial:
This trial aims to evaluate immunogenicity and safety of a 4th vaccination dose of COVID-19 vaccines in older (≥75) subjects having received a primary vaccination series plus a 3rd vaccine dose > 1month ago at the time of enrolment.

Primary endpoints

According to section 4.8.1.1 of the accompanying master protocol:

- Rate of 2-fold antibody titre increase 14 days **after the 4th vaccination dose** measured by quantitative enzyme-linked immunosorbent assay (Anti-RBD-ELISA) against wild-type virus.

Safety endpoints

- Unsolicited AEs until the end of trial.
- Solicited AEs for 7 days after a 4th vaccination dose.
- Rate of severe adverse events (AEs) Grade ≥3 according to the National Cancer Institute Common Toxicity Criteria up to three months after a 4th vaccination dose (Note: Adverse events Grade ≥3 must be reported as SAE by using study specific SAE reporting process, see section 7.3).

Secondary endpoints

- Change in neutralizing antibody titre (Virus Neutralisation Assay) **against wild-type** 14 days after a 4th vaccination dose, to be determined in a subgroup only.

- Change in neutralizing antibody titre (Virus Neutralisation Assay) **against variants of concern** 14 days after a 4th vaccination dose, to be determined in a subgroup only.

- Antibody titre level **12 months** after a 4th vaccination dose measured by a quantitative enzyme-linked immunosorbent assay (anti-RBD-ELISA assay).

- Neutralizing antibody titre (Virus Neutralisation Assay) **against wild-type SARS-CoV-2** at 12 months after a 4th vaccination dose, to be determined in a subgroup only.

- Neutralizing antibody titre (Virus Neutralisation Assay) **against variants of concern** at 12 months after a 4th vaccination dose, to be determined in a subgroup only.

Exploratory endpoints

- Change in cellular immune response measured by qPCR 14 days after 4th booster dose, to be determined in a subgroup only.
- Neutralising capacity measured by neutralising activity against newly emerging variants in bio-banked samples in a subgroup analysis after a 4th vaccination dose, to be determined in a subgroup only.
- Correlates of humoral immune response, cellular immune responses and viral neutralising capacity against SARS-CoV-2 variants of concern (VOCs), to be determined in a subgroup only.

Safety data in Part B

- Solicited AEs (as defined in section 7.1.7) for 7 days after an on-trial 4th vaccination dose will be collected by using a diary (electronic or paper-based). Information collected will be entered into the eCRF by designated study site personnel.
- Unsolicited adverse events will be collected during visits up to the end of trial upon an open question by the investigator to the participant. Those unsolicited AEs graded as severe by the subject will be evaluated by the investigator to define the relationship with administered vaccines. Only those considered related will be accounted for and reported. Serious adverse events (SAEs) will be collected through an open question.

- SADRs will be collected and reported during the visits 4 to 6, reported spontaneously by the subject or elicited after an open question about any event of interest since the previous visit.

8 Description of visits

Patient visits are carried out at the following timepoints (see table below). Subjects will be followed for 1 year after on-study vaccine administration.

Table Visit schedule in Part B

Visit number	**1**	**2**	**3**	**4**	**5**
Procedure	Screening, enrolment, baseline 4th dose	Immune response evaluation	Follow up	Follow up	End of study
Day ± window	0	14 ±2 days after 4th dose	3 months ± 3 days after 4th dose	6 months ± 3 days after 4th dose	12 months ± 3 days after 4th dose
Screening for eligibility					
Informed consent [a]	X				
Demographics and medical history[f]	X				
Eligibility check	X				
Baseline procedures					
Concomitant medication review	X	X			
Physical exam	X	X[b]			
Vital signs	X	X[b]			
Immunogenicity					
Anti-RBD & anti-N IgG (ELISA)	X	X			X
Neutralising activity (wildtype) [c]	X	X			X
Neutralising activity (VOC) [c]	X	X			X
Cellular response (qPCR assay) [c]	X	X			
Biobanking [d]	X	X	X	X	X
IMP administration					
Vaccination[§]	X				
Safety					
AE/SAE [e]	X	X	X	X	X

AE, adverse events; SAE, serious adverse event

a: Informed consent must be obtained before obtaining consent for biobanking and secondary data use, and any other procedure to be undertaken.

b: will be performed at visit 2 only upon SAE

c: samples taken from all subjects, analysis performed in a subgroup only. Analysis will be performed in all samples if additional funding becomes available.

d: for secondary use defined in informed consent; also optional at visit 1 and visit 2: at trial site and upon agreement of trial participant additional blood collection for biobanking of peripheral blood mononuclear cells (PBMC) as per informed consent

e: solicited AEs are recorded by trial participant till Day 7 and records will be collected at visit 2 (Day 14) by trial staff and captured in eCRF; unsolicited AEs are recorded by trial investigator until the end of trial as described in this protocol.

f: medical history includes information on prior SARS-CoV-2 infection and COVID-19 disease if applicable; name of SARS-CoV-2 variant should be documented if known.

§Administration of 4th vaccination dose after blood sampling for immunogenicity and cellular immunity during visit 1. Trial participant is observed for any adverse reaction for at least 15 min or according to standard of care upon vaccination.

Note: The protocol does not foresee withdrawal of subjects with SARS-CoV-2 after randomization (by means of anti-Nucelocapside IgG or external confirmation), they will all be followed up until visit 5 (end of study).

9 Statistical Analysis

In this section the statistical analyses for the primary, secondary and exploratory endpoints are described. This section of the SAP is for Part B of the study, i.e. all statistical analyses described below will be performed using subject data from Part B only (not using subject data from Part A). There will be no blind review of the data before performing the statistical analyses. Individuals in Part A (followed-up as specified in protocol version V04_0) are analysed as outlined in Appendix A of this SAP.

9.1 Analysis populations

According to section 6.1.1 in the accompanying master protocol:

The primary dataset for analysis is derived from the intention-to-treat (ITT; Full Analysis Set, FAS) population. This dataset includes all trial subjects enrolled into the trial and randomised.

The evaluation is carried out strictly in accordance with the allocation by randomisation.

The secondary dataset for analysis is derived from the per-protocol (PP) population. This dataset includes all trial subjects who were treated according to protocol and reached a defined endpoint in the trial.

The tertiary dataset for analysis is the safety population. This population includes all trial subjects who received any IMP or other trial treatment.

Specific to this trial:

All analysis population defined here are specific for part B (using subjects from part B only).

Modified intention-to-treat population (mITT) Population.

> The mITT population includes all subjects who received a study vaccine (as randomised and and administered accordingly) and contributed both pre- and at least one post-vaccination blood sample for immunogenicity testing for which valid results were reported.

Primary Analysis Population

> The primary analysis population is a subset of the modified intention-to-treat population (mITT), consisting of all randomised subjects whose primary endpoint measurement is

available. Please note the primary analysis population will include subjects with an anti-Nucleocapside IgG at visit 2 indicative of SARS-CoV-2 infection.

Per Protocol Population

In the final analysis, protocol deviations will be reviewed to determine which protocol deviations may affect the analysis. The per protocol (PP) population includes all subjects in the mITT subset, but will exclude the following subjects:

- Data from all visits from subjects found to be ineligible at baseline
- Patients who have had a known new SARS infection between baseline and the visit concerned.
- Data from all visits subsequent to major protocol deviations that are considered to affect the outcome
- Data from any visits that occurred substantially out of the foreseen time window

Safety Population

The population for the safety analysis will be all randomised subjects that received a study vaccine. Analyses for the safety population will include safety reported through the end of the study.

9.2 Description of trial subject groups

For Part B, all statistical analyses described below will be performed.

Subjects have been randomised to one of the two intervention groups

- BNT162b2 (Comirnaty) and
- mRNA-1273 (Spikevax).

Patient Cohorts, arm and study **Intervention group in Part B:**

Cohort	Vaccination prior to study entry	Arm	Study intervention: 4th vaccination dose*
Cohort 4	BNT162b2 + BNT162b2 + BNT162b2	7	BNT162b2
		8	mRNA-1273
Cohort 5	BNT162b2 + BNT162b2 + mRNA-1273	9	BNT162b2
		10	mRNA-1273

Cohort 6	mRNA-1273 + mRNA-1273 + mRNA-1273	11	BNT162b2
		12	mRNA-1273
Cohort 7	mRNA-1273 + mRNA-1273 + BNT162b2	13	BNT162b2
		14	mRNA-1273
Cohort 8	ChAdOx-1-S + ChAdOx-1-S + BNT162b2	15	BNT162b2
		16	mRNA-1273
Cohort 9	ChAdOx-1-S + ChAdOx-1-S + mRNA-1273	17	BNT162b2
		18	mRNA-1273

Each of the initial prime boost strategies (see first column in table above) define a cohort of patients. If not otherwise stated, all descriptive analyses described below will be performed overall and separately for cohorts.

Patient demographics and baseline characteristics will be summarised on the modified ITT (mITT) set, overall and by treatment cohort and randomised treatment arms, by means of summary descriptive statistics. Summaries of baseline clinical laboratory values will be presented by overall, treatment arm and by treatment cohort and randomised treatment arms.

For qualitative variables (e.g., gender (biological sex)), absolute and relative frequencies will be calculated per treatment group. Data will be visualised by bar plots. For quantitative data (e.g., age), the number of valid observations, mean, standard deviation, standard error, median, minimum and maximum will be calculated for each treatment group and each time point separately. Data will be visualised by waterfall, boxplots and histograms.

9.3 Primary Endpoint Analysis

All primary analyses will be on the mITT set, consisting of all randomised subjects whose primary endpoint measurement is available. The primary endpoint is the rate π of 2-fold antibody titre increase following the 4th vaccine dose measured by quantitative enzyme-linked immunosorbent assay (Anti-RBD-ELISA) against wildtype virus at 14 days after the 4th vaccine dose (versus immediately before vaccination).

For the binary primary endpoint absolute and frequencies in percent will be calculated for each intervention group in Part B. For each treatment group in part B the corresponding rate together with simultaneous 95% confidence intervals will be calculated. Exact Clopper Pearson confidence

intervals at Bonferroni adjusted level of (1-0.05/2) *100% will be provided (as there are two treatment groups in part B).

9.3.1 Supportive analyses for the primary endpoint

In additional supportive analysis, the primary endpoint will be determined in the same way for each cohort separately, i.e., further supportive analyses will be performed based on different pre-vaccination series. Especially, within each cohort the multiplicity adjustment will be implemented as described above. The data will be visualised with bar charts.

To evaluate whether there is a difference between the two randomised intervention groups in the binary primary endpoint, a Cochrane-Mantel-Haenszel test will be applied stratified by the initial cohort vaccine strategy (cohort 4 to 9).

Furthermore, a logistic regression model will be performed using the primary endpoint as dependent variable. In the logistic regression models intervention group will be used as main factor. Based on the logistic regression model odds ratio (OR) and the corresponding 95% confidence intervals will be reported to evaluate whether there are differences between the two study intervention groups. In the logistics analyses we will also adjust for factors such as initial vaccination regimens (factor cohort), age and sex. We will also account for the different time lags between the 2nd and 3rd doses in further sensitivity analysis, e.g., including the number of days between vaccinations as additional covariate in the analysis models. Furthermore, descriptive tables will be provided stratified by taking into consideration the vaccination regimens.

In addition to the binary primary endpoint, the antibody titre values of visit 1 and 2 will be analysed on the metric scale (see analyses planned for secondary endpoints).

9.3.2 Impact of Covid infections between baseline and day 14

With testing for anti-Nucleocapside IgG at visits 1 (baseline), visit 2 (=d14) and visit 5 (end of study) subjects will be assessed for SARS-CoV-2 infection since randomization. Overall and for all vaccination regimens the proportion of infections between d0 and d14 (based on an anti-Nucleocapside IgG at visit 2 indicative of SARS-CoV-2 infection) will be compared descriptively.

In further logistic regression models the impact of having had a Covid infection between visit 1 and visit 2 on the primary endpoint will be explored by using it as additional factor.

Additionally, a sensitivity analysis will performed on the per protocol analysis set, including only subjects who have been vaccinated and not having had a new infection between visit 1 and visit 2 using the anti-Nucleocapside IgG measurements of visit 1 and 2.

For subjects where the exact timepoint of an externally confirmed infection is available (i.e. subjects reports date of a SARS-CoV-infection confirmed outside study procedures, captured by AE reporting), a further sensitivity analysis will be performed excluding only those patients who had a an externally confirmed infection between randomisation and the observation of the primary endpoint.

9.3.3 Analysis time point for primary endpoint

For the second part B (4th vaccination, cohorts 4-9), the primary endpoint analysis will be triggered as soon as for all patients data collection of the primary endpoint data (14 days after study vaccination) for all patients in part B has been finished. Analysis time (planned): October 2022 (primary endpoint analysis).

9.4 Secondary Endpoint Analyses

Summaries and analysis of the secondary endpoints will be based on the mITT population. If there are protocol deviations which may affect the analysis, a PP analysis may also be performed.

9.4.1 Secondary endpoints:

- Change in neutralizing antibody titre (Virus Neutralisation Assay) against wild-type 14 days after a 4th vaccination dose, to be determined in a subgroup only in which this type of assay has been performed.
- Change in neutralizing antibody titre (Virus Neutralisation Assay) **against variants of concern** 14 days after a 4th vaccination dose, to be determined in a subgroup only in which this type of assay has been performed.
- Antibody titre level **at 12 months** after a 4th vaccination dose measured by a quantitative enzyme-linked immunosorbent assay (anti-RBD-ELISA assay).
- Neutralizing antibody titre (Virus Neutralisation Assay) against wild-type SARS-CoV-2 at 12 months after a 4th vaccination dose, to be determined in a subgroup only in which this type of assay has been performed.
- Neutralizing antibody titre (Virus Neutralisation Assay) **against variants of concern** at 12 months after a 4th vaccination dose, to be determined in a subgroup only in which this type of assay has been performed.

For quantitative data the number of valid observations, mean, standard deviation, standard error, median, minimum and maximum will be calculated for each treatment group and each time point separately. Additionally, descriptive statistics of immunogenicity data may include Geometric Means (GM), Geometric Mean Fold Rise (GMFR) from baseline, and proportion of participants achieving a

pre-specified increase from baseline. Absolute values and the changes will be reported and visualized by individual trajectories of subjects over time via spaghetti plots. Furthermore, mean trajectories (such as means$\pm sem$) and boxplots will be provided.

9.4.2 Non-Inferiority of Geometric Mean titres

The geometric mean titres (GMT) following and the 4th dose vaccination measured by quantitative enzyme-linked immunosorbent assay (Anti-RBD-ELISA) against wildtype virus at 14 days after the 4th dose will be compared between the boosters BNT162b2 and mRNA-1273 computing two-sided 95% confidence intervals for the GMR or differences of GMC on log scale (base 10). Based on the confidence intervals, an equivalence test will be performed. The equivalence margins are the 1.5 to 0.67-fold change between the GMT in the two boost arms corresponding to a margin for the differences of GMC on log scale (base 10) which results in a margin ±0.174. This is suggested in Section 2.3.1 of the EMA/117973/2021 Reflection paper on the regulatory requirements for vaccines intended to provide protection against variant strain(s) of SARS-CoV-2[11]. Because GMT are expected to have a skewed distribution, the log10 scaled GMT values will be compared using a linear model with factors booster (BNT162b2/mRNA-1273) as well as the stratification variables used in the randomisation. As this is a secondary analysis, no adjustment for multiplicity will be made.

9.4.3 Linear Models & Adjustment for Covariates and Mixed Models

When analysing the data from all study visits (e.g., Visit 1, Visit 2 and Visit 5 for immunogenicity data such as Anti-RBD or anti-N IgG), mixed model for repeated measurements (MMRMs) will be applied using subject ID as random factor using an unstructured covariance matrix. The last value before randomisation (baseline) will be used as covariate in the MMRM.

Similarly as described for the logistic regression models for the primary endpoint (see supportive analyses for the primary endpoint), also in the MMRs we adjust for factors/covariates such as initial vaccination regimens (factor cohort), age and sex. We will also address for the different time lags to previous vaccinations, e-g., for the time between the 2nd and 3rd doses and between the 3rd and 4th doses, respectively in further sensitivity analysis, e.g., including the number of days between vaccinations as additional covariate in the analysis models.

The impact of new covid infections after baseline on the secondary endpoints will be explored similarly to the primary endpoint analysis. For example by using an additional factor (New Covid Infections based on anti-Nucleocapside IgG between visits) in the MMRMs and performing analysis on the PP analysis set.

In further secondary analyses, it will be explored whether switching or keeping the same vaccine product for the 4th vaccination (study intervention in B) compared to the 3rd vaccination will have an effect. To evaluate such an impact models will performed using for example the interaction between cohort and study drug or an indicator variable whether the 4th and 3rd vaccination are the same or not.

In addition to using the log10 scaled GMT values (=y) as dependent variable, we will use the relative change compared to the baseline value as dependent variable. The relative change is defined as (Y_visit-Y_baseline) /Y_baseline. The change might will be presented as percentage change.

The secondary analyses will be performed overall and for cohorts separately.

9.5 Exploratory analyses

Additional to the primary and secondary immunogenicity endpoints, exploratory endpoints include:

- Change in cellular immune response (CD4+ and CD8+ T cell response) measured by qPCR 14 days after 4th booster dose, to be determined in a subgroup only in which this type of assay has been performed.
- Neutralizing antibody titre (Virus Neutralisation Assay) against newly emerging variants in bio-banked samples in a subgroup analysis after 4th vaccination dose. To be determined in a subgroup only in which this type of assay has been performed.
- Correlates of humoral immune response, cellular immune response and viral neutralising capacity against SARS-CoV-2 variants of concern (VOCs), to be determined in a subgroup only in which this type of assay has been performed.

Different combinations of vaccines (1st, 2nd, 3rd and the on-study 4th vaccine dose) will be assessed for equivalence in terms of humoral and/or cellular immune response against VOC compared to approved homologous COVID-19 vaccination schedules. The analysis strategy will be performed as described for the primary and secondary endpoints (see sections 9.1.3 and 9.1.4)

Furthermore, exploratory comparisons of the primary endpoint as well as the humoral and/or cellular immune response against VOC will be performed between cohorts as well as between the treatment groups within cohorts.

<u>Correlations between different antibody titre values</u>

To visualize whether there is a correlation between laboratory and demographic data at specific visits, such metric data will be visualised via scatterplots. Correlation coefficients by Pearson and

Spearman will be reported. The correlation analysis will be performed for both absolute values and changes (=difference between two visits).

Optional analysis:

As an option it is planned to perform comparisons with the control group from EU-COVAT subprotocol with the EudraCT no.: 2021-004889-35. The control group of EU-COVAT subprotocol EudraCT no. 2021-004889-35 will be only utilised if the sample size in the age group of interest is sufficiently large. Otherwise, the data of the control group will be reported only descriptively. These analyses will be performed similar to the analysis described in 9.1.4. and 9.1.6. If such analyses are actually performed the details of the analysis will be stated in the report.

9.6 Data Transformations

The data will be visualised by histograms. Skewed laboratory data will be transformed using appropriate functions such a log, log10 or sqrt. The transformations will be noted in the final report.

For laboratory values:

For values below the Lower Limit of Detection (LLOD) we will impute a value equivalent to LLOD/2 if such a value is marked by the laboratory and the LLOD value provided. Laboratory values that are reported as above the LLOD but below the Lower Limit of Quantification (LLOQ) will be kept as reported by the corresponding Laboratory if actual numerical values are provided. If actual values below the LLOQ are not provided, the observations will be replaced with a value equivalent LLOQ/2. Values that are greater than the upper limit of quantification (ULOQ) will be kept when actual values are reported from the corresponding laboratory, otherwise the ULOQ if provided by the laboratory and the value is marked.

9.7 SARS-CoV-2 infection since randomization

With testing for anti-Nucleocapside IgG at visits 1 (baseline), visit 2 (=d14) and visit 5 (end of study) subjects will be assessed for SARS-CoV-2 infection since randomization. For all vaccination regimens the proportion of infections between d0, d14 and visit 5 (based on an anti-Nucleocapside IgG at visit 2 indicative of SARS-CoV-2 infection) will be compared descriptively reporting absolute numbers and percentages.

9.8 Multiplicity adjustment

The different pre-study vaccine combinations are considered as inferentially independent, as the prior vaccination strategy cannot be influenced and there will be no extrapolation from one cohort to another one for the primary analysis. Therefore, the multiplicity adjustment concerning the primary endpoint will be applied within each cohort, but there will be no further adjustment for having several cohorts within the accompanying master protocol. The comparison of treatment group is of secondary purposes, which are not subject of further multiplicity adjustments.

9.9 Subgroup analyses

Subgroup analyses will be performed by gender and documented history of prior COVID-19 infection (yes/no), respectively.

As the sample size was fixed for part B overall, but not for the individual cohorts, the observed sample sizes in the cohorts might differ substantially. Therefore, cohorts might be merged based on the final sample sizes observed and scientific reasoning.

Only for a subgroup of subjects certain endpoints as defined in the trial protocol (see variables in first column of the visit schedule marked with subindex c) will be determined. Currently, in a first step this will include the first 200 enrolled subjects across Part A und B, but may be expanded later if additional funding becomes available.

9.10 Interim Analysis

An interim analysis will be performed as soon as 50% of participants have been recruited within Part B. In the interim analysis, Haybittle–Peto boundaries of 0.0001 will be used to calculate the confidence intervals for the primary analysis in each cohort. There will be no stopping because of differences in the primary endpoint analysis at interim analysis..

If a treatment arm is dropped due to safety concerns, a sample size re-allocation to the remaining arms might be considered. However, in this case the overall sample is still fixed as initially planned, i.e., the total sample size over all cohorts in Part B is bounded by 550 patients in total

The interim analysis will allow for a sample size reassessment in the interim analysis, e.g., to re-estimate the standard deviation. If a sample size reassessment is performed, the conditional error principle is implemented to control the FWER for the primary endpoint.

Note: In case enrolment is much faster than assumed at the time of trial design, the interim analysis may be omitted.

10 Safety Analysis

Summaries and analysis of safety data will be presented for the Safety Analysis Population.

An adverse event (AE) is any untoward medical occurrence in a trial subject administered an IMP. There does not necessarily have to be a causal relationship with this treatment. The adverse event may be, but is not restricted to: a new illness, worsening of a sign or symptom following trial-specific treatment (here 4th vaccination dose), the clinically significant abnormal results of an examination (e.g., laboratory findings, electrocardiogram) or deterioration of a pre-existing medical condition, or a combination of two or more of these factors, as applicable.

AEs will be documented starting from baseline visit and until the last scheduled visit after the 4th vaccine dose unless otherwise defined in the sub-protocol specific visit schedule.

The safety endpoints includes

- Unsolicited AEs until the end of trial.
- Solicited AEs for 7 days after a 4th vaccination dose.
- Rate of serious adverse events (SAEs) Grade ≥3 according to the National Cancer Institute Common Toxicity Criteria up to three months after a 4th vaccination dose.

Listing will be provided on a subject level reporting the severity and relationship to study vaccination (related / unrelated). Absolute and numbers in percent will be given per intervention group. Different types of AEs will be grouped. Additionally the safety data will be reported descriptively for each intervention and cohort separately.

11 Software program(s):

All statistical analyses will be conducted with statistical software like SAS 9.4. (or higher) and R 3.6.3. (or higher).

12 Sample size calculation

The number needed has been calculated at 550 for part B (i.e., 600 overall for Part A and B, and including a potential dropout rate of 8-10% for Part B).

12.1 Sample size calculation with multiplicity adjustment:

Sample size calculation has been carried out for the primary endpoint for the rate π of 2-fold antibody titre increase following 4th dose vaccination. As two-sided simultaneous 95% confidence intervals for this rate should be calculated separately for each randomized group in Part B a Bonferroni adjustment was used in the sample size calculation accordingly.

When the sample size is 250 per randomized group, two-sided simultaneous 95% confidence intervals (with Bonferroni adjustment for 2 simultaneous confidence intervals in a cohort) for a proportion using the large sample normal approximation will extend no more than ±7.1% (percentage points) from the observed proportion. E.g., if the observed proportion is 50% (where the confidence interval is widest), the confidence interval ranges from about 42.9% to 57.1%.

To adjust for potential dropouts in part B of about 8-10%, the total sample size for part B (=4th vaccination and Cohorts 4-9) was set to 550.

In the table below the precision for the simultaneous two-sided 95%-confidence intervals (with the same assumptions as described above) is shown for varying sample sizes. This shows which precision could be achieved in the different cohorts based on the previous vaccination strategy (see also inclusion criteria for Part B).

Sample Size, n	250	200	150	125	90	50	40
Expected Proportion, π	0.500	0.500	0.500	0.500	0.5	0.500	0,5
Distance from Proportion to Limit, ω	0.071	0.079	0.092	0.100	0.118	0.158	0.177

Also, the sample size of n=550 in part B shall provide adequate power for comparison in GMT (Geometric mean titre values) between the two randomised treatment arms in aged patients, e.g., comparison of different boosting strategies depending on the initial vaccination. E.g., demonstration of equivalent immune response after heterologous boosting, in comparison with the homologous approach, will facilitate vaccination programs when vaccine availability is an issue.

	APPENDIX A: Statistical Analysis Plan for part A
Title of Trial	A Multinational, Phase 2, Randomised, Adaptive Protocol to Evaluate Immunogenicity and Reactogenicity of Different COVID-19 Vaccines Administration in Older Adults (≥75) Already Vaccinated Against SARS- CoV-2 (EU-COVAT-1 Aged)
Short Title	EU-COVAT-1 Aged
Sponsor Trial No.	uni-koeln-4602
EudraCT No..	2021-004526-29

PLEASE NOTE:

The APPENDIX A of this SAP refers to Part A.

Part A of the present trial in which individuals received a 3rd vaccination (first booster) was closed to further recruitment as of January 13, 2022. With the massive roll-out of booster campaigns throughout Europe, Part A was abandoned because of a poor recruitment rate.

Individuals in Part A are followed-up as specified in protocol version V04_0 Therefore, the overview on part A and any references in appendix A to parts in the study protocol refer protocol version V04_0 (which covers Part A).

Sponsor	University of Cologne Represented by: Prof. Dr. med. Oliver Cornely (Principal Coordinating Investigator, PCI) Department for Internal Medicine I University Hospital Cologne Kerpener Strasse 62 50937 Cologne Germany
Principal Coordinating Investigator	See above National representatives are detailed in a separate list for this multinational trial.
Title of the clinical trial	**A Multinational, Phase 2, Randomised, Adaptive Protocol to Evaluate Immunogenicity and Reactogenicity of Different COVID-19 Vaccines Administration in Older Adults (≥75) Already Vaccinated Against SARS-CoV-2 (EU-COVAT-1 Aged)**
Indication	COVID-19 vaccination
Phase	Phase ll clinical trial

Type of trial, trial design, methodology	**Please note: This trial ("*EU-COVAT-1_Aged")* is a sub-protocol embedded within the *EU-COVAT* master protocol.** This is a randomised controlled, adaptive, multicentre Phase II protocol evaluating different booster strategies in individuals aged 75 years and older already vaccinated against SARS-CoV-2. This trial foresees testing of different vaccines as a third/booster dose for comparative assessment of their immunogenicity and safety against SARS-CoV-2 and SARS-CoV-2 variants in the elderly, a usually neglected population. Different vaccines and extended follow-up visits can be added through amendments of this sub-protocol. As stated in the *EU-COVAT* master protocol, this trial, i.e., the *EU-COVAT-1 Aged study,* implements a specific safety monitoring strategy (see below). **Randomisation** Subjects will be randomised to one of the arms planned in an equal allocation ratio. The allocation ratio is in each of the three cohorts 1:1 to the two arms BNT162b2 (Comirnaty) and mRNA-1273 (Spikevax). Cohorts and arms can be withdrawn or added as deemed necessary according to the criteria specified in this protocol. **Blinding** No blinding is foreseen in this trial
Number of subjects	The number needed has been calculated at 100 subjects per arm (i.e., 600 in total, including an assumed 10% of dropouts, for the 6 planned initial arms). **Sample size calculation with multiplicity adjustment within each cohort** When the sample size is 90 per group (without dropouts, i.e., 100 including 10% dropouts), two-sided simultaneous 95% confidence intervals (with Bonferroni adjustment for 2 simultaneous confidence intervals within a cohort) for a proportion using the large sample normal approximation will extend no more than ±12% (percentage points) from the observed proportion. E.g., if the observed proportion is 50% (where the confidence interval is widest), the confidence interval ranges from about 38% to 62%.
Primary trial objective	To evaluate immune response against wild-type SARS-CoV-2 of different booster strategies in elderly subjects (*≥75 years old)* already fully vaccinated against SARS-CoV-2.

Secondary trial objectives	• To compare the humoral immune response against wild-type SARS-CoV-2 between treatment arms within each cohort following 3rd vaccination dose in elderly individuals (≥75 years) already vaccinated against SARS-CoV-2. • To compare the humoral immune response against wild-type SARS-CoV-2 between cohorts following 3rd vaccination dose in elderly individuals (≥75 years) already vaccinated against SARS-CoV-2. • To evaluate immune response against SARS-CoV-2 variants of concern of different booster strategies in elderly individuals (≥75 years) already fully vaccinated against SARS-CoV-2. • To assess the CD4+ and CD8+ T cell response of different booster strategies in elderly individuals (≥75 years) already vaccinated against SARS-CoV-2. • To evaluate the long-term humoral immune response of different booster strategies in individuals already fully vaccinated against SARS-CoV-2.
Safety objectives	• To assess the safety of different booster strategies in elderly individuals (≥75 years) already vaccinated against SARS-CoV-2.

Study end points	**Primary end point:** • Rate of 2-fold antibody titre increase following 3rd dose vaccination measured by quantitative enzyme-linked immunosorbent assay (Anti-RBD-ELISA) against wildtype virus 14 days after 3rd dose. **Secondary end points:** <u>Safety:</u> • Unsolicited AEs until the end of trial. • Solicited AEs for 7 days after 3rd dose. • Rate of serious adverse events (SAEs) Grade ≥3 according to the National Cancer Institute Common Toxicity Criteria up to three months after 3rd dose. <u>Immunogenicity:</u> • Antibody titre increase following 3rd dose measured by neutralising activity against wildtype virus (Virus Neutralisation Assay) in a subgroup 14 days after 3rd dose. <u>Immunogenicity against variants:</u> • Change in neutralising capacity measured by neutralising activity against variants of concern (Virus Neutralisation Assay) in a subgroup 14 days after 3rd dose. <u>Long-term immunity:</u> • Antibody titre level following 3rd dose measured by a quantitative enzyme-linked immunosorbent assay (anti-RBD-ELISA assay) 12 months after 3rd dose. • Antibody titre level following 3rd dose measured by neutralising activity against wildtype virus (Virus Neutralisation Assay) in a subgroup 12 months after 3rd dose. • Change in neutralising capacity 12 months after 3rd dose measured by neutralising activity against variants of concern (Virus Neutralisation Assay) in a subgroup of subjects. **Exploratory endpoints:** • Change in cellular immune response measured by qPCR 14 days after 3rd dose in a subgroup analysis. • Neutralising capacity measured by neutralising activity against newly emerging variants in bio-banked samples in a subgroup analysis.

	• Correlates of humoral immune response, cellular immune responses and viral neutralising capacity against SARS-CoV-2 variants of concern (VOCs).
Diagnosis and Principal inclusion and exclusion criteria	**Medical condition or disease to be investigated:** Prevention of COVID-19 infection. **Principal inclusion criteria:** • Elderly (≥75 years old). • Already fully vaccinated adults. • Primary vaccination (1st and 2nd dose) using BNT162b2, mRNA-1273 or ChAdOx-1-S). • No contra-indication against any of the vaccine products in the trial at time of enrolment. **Stratification and subpopulations:** • Vaccine product used for primary vaccination (homologous 1st and 2nd dose using BNT162b2, mRNA-1273 or ChAdOx-1-S). • Gender. • Documented history of prior COVID-19 infection (if more than 3 months ago prior to enrolment; yes/no). **Principal exclusion criteria**: • Primary vaccination performed with different vaccine products as sole (e.g., COVID-19 Vaccine Janssen) or, 1st and 2nd vaccination doses (heterologous vaccination scheme). • Any contraindication against any of the vaccines at time of enrolment including current SARS-CoV-2 infection or proven in the last 3 months. • Immunocompromised status.
Name of investigational medicinal product (IMP)	• BNT162b2 (Tradename Comirnaty) • mRNA-1273 (Tradename Spikevax)

<table>
<tr><td>Investigational medicinal product – dosage and method of administration</td><td>Intervention: mRNA-based booster strategies following different prime boost vaccination strategies.
<table>
<tr><th>Cohort</th><th>Prime strategy</th><th>Intervention Arm</th><th>Intervention: Third dose</th><th>Interval</th></tr>
<tr><td rowspan="2">Cohort 1</td><td rowspan="2">BNT162b2 - BNT162b2</td><td>1</td><td>BNT162b2</td><td rowspan="6">9 ± 3 months from date of 2nd vaccine dose upon enrolment</td></tr>
<tr><td>2</td><td>mRNA-1273</td></tr>
<tr><td rowspan="2">Cohort 2</td><td rowspan="2">mRNA-1273 - mRNA-1273</td><td>3</td><td>BNT162b2</td></tr>
<tr><td>4</td><td>mRNA-1273</td></tr>
<tr><td rowspan="2">Cohort 3</td><td rowspan="2">ChAdOx-1-S - ChAdOx-1-S</td><td>5</td><td>BNT162b2</td></tr>
<tr><td>6</td><td>mRNA-1273</td></tr>
<tr><td>Control</td><td></td><td colspan="3">Control arm of the EU-COVAT subprotocol EudraCT no. 2021-004889-35, a separate sub-protocol embedded within the EU-COVAT master protocol*</td></tr>
</table>
*The control arm will be used for a descriptive comparison.</td></tr>
<tr><td>IMP or therapy used as a comparator – dosage and method of administration</td><td>No vaccination in the control group of the EU-COVAT subprotocol EudraCT no. 2021-004889-35, a separate sub-protocol embedded within the EU-COVAT master protocol</td></tr>
<tr><td>Duration of treatment:</td><td>Treatment consists of a single 3rd boost dose of each vaccine included in this protocol. Follow up of subject included will last for 12 months after boost dose.</td></tr>
<tr><td>Time plan</td><td>
<table>
<tr><td>First patient first visit (FPFV):</td><td>October 2021</td></tr>
<tr><td>Last patient first visit (LPFV):</td><td>March 2022</td></tr>
<tr><td>Last patient last visit (LPLV):</td><td>April 2023</td></tr>
<tr><td>Analysis</td><td>April 2022 (Primary endpoint analysis)</td></tr>
<tr><td>End of study definition</td><td>The end of study will be on the day of database lock.</td></tr>
<tr><td>End of trial</td><td>May 2023</td></tr>
<tr><td>Final study report:</td><td>July 2023</td></tr>
</table>
</td></tr>
</table>

Statistician	Assoc. Prof. Priv.-Doz. Dr. Franz König Center for Medical Statistics, Informatics and Intelligent Systems Medical University of Vienna Spitalgasse 23, 1090 Vienna Austria
Statistical methods	**Firstly,** the rates of 2-fold increase in antibody titre with simultaneous 95% confidence intervals (adjusted for the 6 comparisons) will be computed. **Secondly,** each of the three prime strategies (BB, MM, AA) define a cohort of study participants. For each cohort, we compare the boosters B and M and compute a two-sided confidence interval. Based on these we test for equivalence of one of the 3rd vaccination dose (based on the equivalence margin as outlined in the Reflection Paper of EMA). **Thirdly,** the heterologous prime-boost COVID-19 vaccination will be assessed for equivalence in humoral immune response against VOC compared to approved homologous COVID-19 vaccination. **The primary endpoint analysis** will be performed as soon as single cohorts have completed day 14 of the visit schedule. The primary endpoint analysis will be triggered as soon as for all patients the primary endpoint (14 days after 3rd vaccination) has been observed. **Safety analysis.** All clinical safety data will be listed by participant, time from vaccination dose and treatment arm. Continuous variables will be summarised using sample size (N), mean, standard deviation, median, minimum, and maximum. Frequency counts will be reported for categorical data. A Data and Safety Monitoring Committee (DSMC) will review data for decisions on arms incorporation and withdrawal and will ensure the appropriate oversight and monitoring in conducting the clinical trial. An interim analysis will be implemented if deemed necessary, when 300 participants completed the primary endpoint assessment.

GCP compliance	The present trial will be conducted in accordance with the valid versions of the trial protocol and the internationally recognised Good Clinical Practice Guidelines (ICH-GCP), including archiving of essential documents.
Financing	European Commission

13 General aspects of trial design

According to section 4.1 of the accompanying master protocol:

This is a randomised, adaptive, multicentre, Phase II master protocol evaluating different booster strategies in individuals already vaccinated against SARS-CoV-2. This protocol allows testing of different booster strategies to assess their immunogenicity and safety against SARS-CoV-2 and its variants. Different trial populations, different vaccines, additional doses and vaccination time points, and extended follow-up visits can be added throughout amendments of the master protocol. The master protocol contains a common control group that does not receive a 3rd vaccination dose. This control group is used for exploratory comparison as applicable. The trial implements a specific safety monitoring strategy for this control group.

13.1 Specific to this trial:

This is a randomised controlled, adaptive, multicentre Phase II protocol evaluating the immunogenicity and reactogenicity of different booster strategies in individuals ≥75 years already vaccinated against SARS-CoV-2. This trial foresees testing of different vaccines as booster/3rd dose to assess their immunogenicity and safety against SARS-CoV-2 and SARS-CoV-2 variants in aged population, a usually neglected population. Different vaccines and extended follow-up visits can be added by means of amendments to this sub-protocol. As stated in the master protocol, this trial implements a specific safety monitoring strategy for the control group (see below).

Subjects in each cohort will be randomised to one of the arms planned in an equal allocation ratio. In each cohort, the arms foreseen at this moment are 2; therefore, the allocation ratio within in each cohort will be 1:1.

Arms can be withdrawn or added as deemed necessary according to the criteria specified in this protocol.

Figure 1 shows a schematic flow chart of the trial design planned for part A.

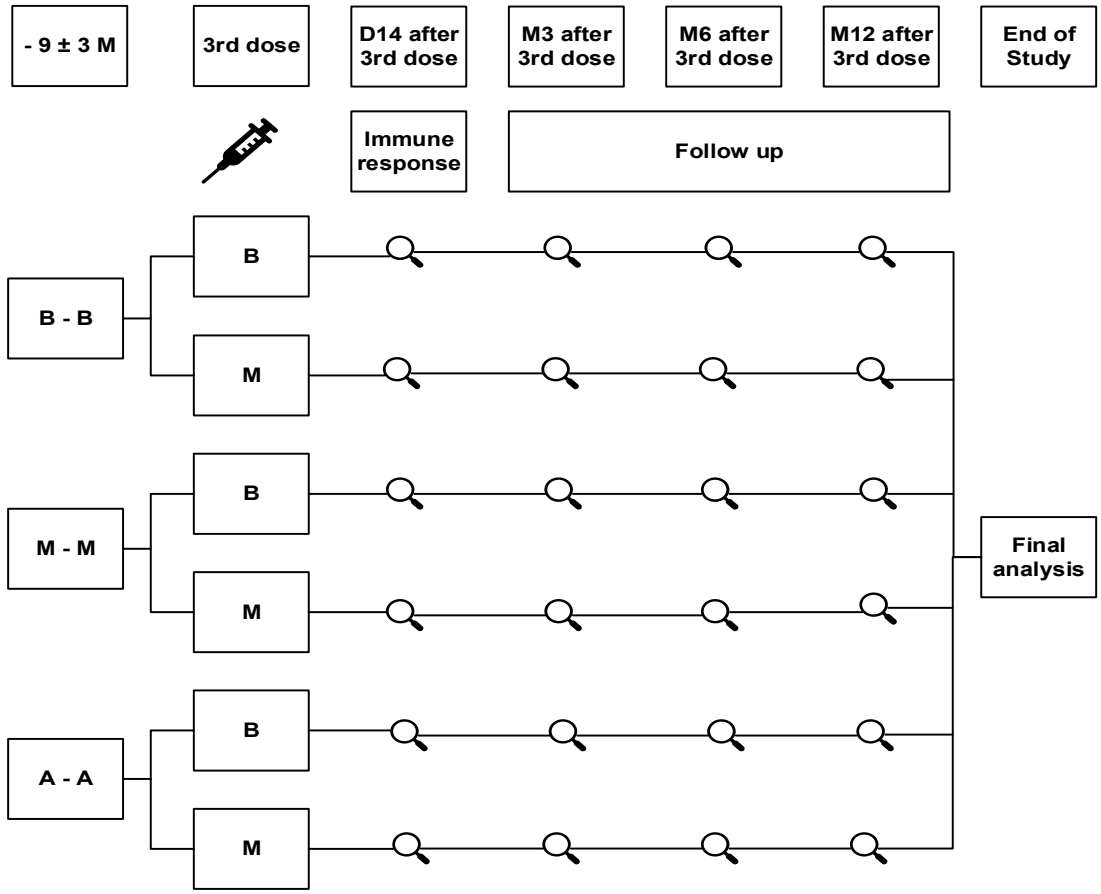


Figure 2: Flow chart of the trial design

Legend: A = Astra Zeneca vaccine ChAdOx-1-S, B = BioNTech vaccine Comirnaty, M = Moderna vaccine Spikevax; D14 = day 14, M3/6/12 = month 3/6/12

14 Discussion of trial design

According to section 4.2 of the accompanying master protocol:

Trials under the master protocol allow testing of different booster strategies to assess their immunogenicity and safety against SARS-CoV-2 and its variants. Different trial populations, different vaccines, additional doses and vaccination time points, and extended follow-up visits can be added throughout amendments of the master protocol or trials under the master protocol.

Specific to this trial:

This trial is a sub-protocol embedded within the master protocol EU-COVAT: A Randomised, Controlled, Adaptive, Multicentre, Phase II Master Protocol Evaluating Different Booster Strategies in Individuals Already Vaccinated Against SARS-CoV-2.

This is a randomised controlled, adaptive, evaluator-blinded multicentre Phase II protocol evaluating immunogenicity and reactogenicity of different booster strategies in individuals 75 years and older already vaccinated against SARS-CoV-2. This trial foresees testing of different vaccines as a third booster dose (homologous and heterologous) for comparative assessment of their immunogenicity and safety against SARS-CoV-2 and SARS-CoV-2 variants in aged population, a usually neglected population. Different vaccines and extended follow-up visits can be added by means of amendments to this trial. As stated in the master protocol, this trial implements a specific safety monitoring strategy for a control group as implemented in the EU-COVAT subprotocol with the EudraCT no.: 2021-004889-35, a separate sub-protocol embedded within the EU-COVAT master protocol (see below). This control group is used for exploratory comparison.

Vaccines used in this trial will be those already marketed and in their commercial presentation. The sample size planned is adequate for a precise enough estimation of the immune response after a 3rd vaccination dose. Also, it will provide adequate power for comparison in GMT (Geometric mean titre values) between the two randomised treatment arms in aged patients, e.g., comparison of homologous versus heterologous boosting strategies depending on the initial vaccination. Demonstration of equivalent immune response after heterologous boosting, in comparison with the homologous approach, will facilitate vaccination programs when vaccine availability is an issue.

15 Assignment of trial subjects to treatment groups

According to section 4.7.5 of the accompanying master protocol:

Subject fulfilling selection criteria will be randomised to the different treatment arms in the trial through a central procedure. Randomisation will be stratified according to:

- Vaccine product used for primary vaccination (detailed in the concerned sub-protocol).
- Trial Site.
- Gender (female/male).
- Immune status (competent, immunocompromised as applicable in the concerned sub-protocol).
- Documented history of prior COVID-19 infection (none, asymptomatic, symptomatic/pre-vaccination or post-vaccination).

The participants are randomised into the study arms as defined in each sub-protocol. Randomisation will be implemented by a 24/7-Internet service (ALEA 17.1, FormsVision BV, Abcoude, NL) and prepared centrally by the Institute of Medical Statistics and Computational Biology (IMSB) at the University of Cologne.

Specific to this trial:

Subjects fulfilling selection criteria will be randomised to the different vaccines (3rd dose) in the trial through a central procedure. Patients will be randomised in a 1:1 ratio to one of the two treatment groups:

- 3rd vaccination with BTN162b2 (Comirnaty).
- 3rd vaccination with mRNA-1273 (Spikevax).

Randomisation will be stratified as described above according to the accompanying master protocol with the following details for this trial:

Separate randomisation lists will be used for each cohort defined by the vaccine product used for the initial primary vaccination (either 2x BNT162b2; 2x mRNA-1273; or 2x ChAdOx-1-S).

In this trial, there is no stratification in the randomisation

- according to the status of immune competency of the trial subject.
- according to trial site.

16 Statistical methods and sample size calculation

In this section of the appendix A the statistical analyses for the primary, secondary and exploratory endpoints are described for Part A. There will be no blind review of the data before conducting the statistical analysis. The appendix of the SAP is for Part A of the study, i.e. all statistical analyses described below will be performed using subject data from Part A.

16.1 Analysis populations

The primary dataset for analysis is derived from the intention-to-treat (ITT; Full Analysis Set, FAS) population. This dataset includes all trial subjects enrolled into the trial and randomised.

The evaluation is carried out strictly in accordance with the allocation by randomisation.

The secondary dataset for analysis is derived from the per-protocol (PP) population. This dataset includes all trial subjects who were treated according to protocol and reached a defined endpoint in the trial.

The tertiary dataset for analysis is the safety population. This population includes all trial subjects who received any IMP or other trial treatment.

Specific to this trial:

All analysis population defined here are specific for part A (using subjects from part A only).

Modified intention-to-treat population (mITT) Population.

The mITT population includes all subjects who received a study vaccine (as randomised and and administered accordingly) and contributed both pre- and at least one post-vaccination blood sample for immunogenicity testing for which valid results were reported.

Primary Analysis Population

The primary analysis population is a subset of the modified intention-to-treat population (mITT), consisting of all randomised subjects whose primary endpoint measurement is available. Please note the primary analysis population will include subjects with an anti-Nucleocapside IgG at visit 2 indicative of SARS-CoV-2 infection.

Per Protocol Population

In the final analysis, protocol deviations will be reviewed to determine which protocol deviations may affect the analysis. The per protocol (PP) population includes all subjects in the mITT subset, but will exclude the following subjects:

- Data from all visits from subjects found to be ineligible at baseline
- Patients who have had a known new SARS infection between baseline and the visit concerned.
- Data from all visits subsequent to major protocol deviations that are considered to affect the outcome
- Data from any visits that occurred substantially out of the foreseen time window

Safety Population

The population for the safety analysis will be all randomised subjects that received a study vaccine. Analyses for the safety population will include safety reported through the end of the study.

16.2 Description of trial subject groups

For Part A, all statistical analyses described below will be performed.

Each of the three prime boost strategies (BB, MM, AA) define a cohort of patients. Due to the termination of part A, the analysis will be done mainly overall (pooling the data of all three cohorts). Additionally the analyses described below will be performed separately in each of the cohorts descriptevely.

Within each cohort subjects are randomised to one of the two intervention groups BNT162b2 (Comirnaty) and mRNA-1273 (Spikevax).

Patient demographics and baseline characteristics will be summarised on the modified ITT (mITT) set, overall and by treatment cohort and randomised treatment arms, by means of summary descriptive statistics.

For qualitative variables (e.g., gender (biological sex)), absolute and relative frequencies will be calculated per treatment group. Data will be visualised by bar plots. For quantitative data (e.g., age), the number of valid observations, mean, standard deviation, standard error, median, minimum and

maximum will be calculated for each treatment group and each time point separately. Data will be visualised by waterfall, boxplots and histograms.

16.3 Primary analysis

All primary analyses will be on the mITT set. The primary endpoint is the rate π of 2-fold antibody titre increase following 3rd dose vaccination measured by quantitative enzyme-linked immunosorbent assay (Anti-RBD-ELISA) against wildtype virus at 14 days after 3rd dose (versus immediately before vaccination).

For the binary primary endpoint absolute and frequencies in percent will be calculated per treatment group (overall and for each cohort). Per treatment group the corresponding rate together with simultaneous 95% confidence intervals will be calculated. A multiplicity adjustment will be implemented. Exact Clopper Pearson confidence intervals at Bonferroni adjusted level of (1-0.05/2)*100% will be provided (as there are two treatment groups in part A).

The data will be visualised with bar charts.

Supportive analyses for the primary endpoint

In additional supportive analysis, the primary endpoint will be determined descriptively in the same way for each cohort separately (for cohorts with sufficient sample sizes), i.e., further supportive analyses will be performed based on different pre-vaccination series. The data will be visualised with bar charts.

To evaluate whether there is a difference between the two randomised intervention groups in the binary primary endpoint, a Cochrane-Mantel-Haenszel test will be applied stratified by the initial cohort vaccine strategy (cohort 1 to 3).

Furthermore, a logistic regression model will be performed using the primary endpoint as dependent variable. In the logistic regression models intervention group will be used as main factor. Based on the logistic regression model odds ratio (OR) and the corresponding 95% confidence intervals will be reported to evaluate whether there are differences between the two study intervention groups. In the logistic regression analyses we will also adjust for factors such as initial vaccination regimens (factor cohort), age and sex. We will also account for the different time lags between vaccinations, i.e., including the number of days between vaccinations as additional covariate in the analysis models. Furthermore, descriptive tables be will provided stratified by taking into consideration the vaccination regimens.

In addition to the binary primary endpoint, the antibody titre values of visit 1 and 2 will be analysed on the metric scale (see analyses planned for secondary endpoints).

16.4 Secondary analyses

Summaries and analysis of the secondary endpoints will be based on the mITT population. If there are protocol deviations which may affect the analysis, a PP analysis may also be performed.

For quantitative data the number of valid observations, mean, standard deviation, standard error, median, minimum and maximum will be calculated for each treatment group and each time point separately. Additionally, descriptive statistics of immunogenicity data may include Geometric Means (GM), Geometric Mean Fold Rise (GMFR) from baseline, and proportion of participants achieving a pre-specified increase from baseline. Absolute values and the changes will be reported and visualized by individual trajectories of subjects over time via spaghetti plots. Furthermore, mean trajectories (such as means$\pm sem$) and boxplots will be provided.

Non-Inferiority of Geometric Mean titres

The geometric mean titres (GMT) following 3rd dose vaccination measured by quantitative enzyme-linked immunosorbent assay (Anti-RBD-ELISA) against wildtype virus at 14 days after 3rd dose will be compared between the boosters BNT162b2 and mRNA-1273 computing two-sided 95% confidence intervals for the GMR or differences of GMC on log scale (base 10). Based on the confidence intervals, an equivalence test will be performed. The equivalence margins are the 1.5 to 0.67-fold change between the GMT in the two boost arms corresponding to a margin for the differences of GMC on log scale (base 10) which results in a margin ±0.174. This is suggested in Section 2.3.1 of the EMA/117973/2021 Reflection paper on the regulatory requirements for vaccines intended to provide protection against variant strain(s) of SARS-CoV-2[11]. Because GMT are expected to have a skewed distribution, the log10 scaled GMT values will be compared using a linear model with factors booster (BNT162b2/mRNA-1273) as well as the stratification variables used in the randomisation. As this is a secondary analysis, no adjustment for multiplicity will be made.

Linear Models & Adjustment for Covariates and Mixed Models

When analysing the data from all study visits (e.g., Visit 1, Visit 2 and Visit 5 for immunogenicity data such as Anti-RBD or anti-N IgG), mixed model for repeated measurements (MMRMs) will be applied using subject ID as random factor using an unstructured covariance matrix. The last value before randomisation (baseline) will be used as covariate in the MMRM.

Similarly as described for the logistic regression models for the primary endpoint (see supportive analyses for the primary endpoint), also in the MMRs we adjust for factors/covariates such as initial vaccination regimens (factor cohort), age and sex. We will also address for the different time lags between the previous vaccinations, i.e., including the number of days between vaccinations as additional covariate in the analysis models.

The impact of new covid infections after baseline on the secondary endpoints will be explored similarly to the primary endpoint analysis. For example by using an additional factor (New Covid Infections based on anti-Nucleocapside IgG between visits) in the MMRMs and performing analysis on the PP analysis set.

In addition to using the log10 scaled GMT values (=y) as dependent variable, we will use the relative change compared to the baseline value as dependent variable. The relative change is defined as (Y_visit-Y_baseline) /Y_baseline. The change might will be presented as percentage change.

The secondary analyses will be performed overall.

16.5 Exploratory analyses

Heterologous prime-boost COVID-19 vaccination will be assessed for equivalence in terms of humoral immune response against VOC compared to approved homologous COVID-19 vaccination. Furthermore, exploratory comparisons of the primary endpoint as well as the humoral immune response against VOC will be performed between cohorts as well as between the treatment groups within cohorts and the control group from EU-COVAT subprotocol with the EudraCT no.: 2021-004889-35.

Optional Analysis

As an option it is planned to perform comparisons with the control group from EU-COVAT subprotocol with the EudraCT no.: 2021-004889-35. The control group of EU-COVAT subprotocol EudraCT no. 2021-004889-35 will be only utilised if the sample size in the age group of interest is sufficiently large. Otherwise, the data of the control group will be reported only descriptively. These analyses will be performed similar to the analysis as described above. If such analyses are actually performed the details of the analysis will be stated in the report.

16.6 Multiplicity adjustment

Part A is considered exploratory due to the early termination. Therefore, the multiplicity adjustment concerning the primary endpoint will be applied when presenting the confidence interval for the primary endpoint for the two randomized groups. But there will be no further adjustment for having several cohorts within the accompanying master protocol.

For the initial sample size calculation and protocol the cohorts defined by three prime boost strategies (BB, MM, AA) have been considered as inferentially independent, as the prior vaccination strategy cannot be influenced and there will be no extrapolation from one cohort to another one for the primary analysis.

16.7 **Subgroup analyses**

Subgroup analyses will be performed by gender and documented history of prior COVID-19 infection (yes/no), respectively.

As part A was prematurely closed, the observed sample sizes in the cohorts 1-3 might differ substantially. Therefore, cohorts might be merged based on the final sample sizes observed and scientific reasoning.

16.8 Data Transformations

The data will be visualised by histograms. Skewed laboratory data will be transformed using appropriate functions such a log, log10 or sqrt. The transformations will be noted in the final report.

For laboratory values:

For values below the Lower Limit of Detection (LLOD) we will impute a value equivalent to LLOD/2 if such a value is marked by the laboratory and the LLOD value provided. Laboratory values that are reported as above the LLOD but below the Lower Limit of Quantification (LLOQ) will be kept as reported by the corresponding Laboratory if actual numerical values are provided. If actual values below the LLOQ are not provided, the observations will be replaced with a value equivalent LLOQ/2. Values that are greater than the upper limit of quantification (ULOQ) will be kept when actual values are reported from the corresponding laboratory, otherwise the ULOQ if provided by the laboratory and the value is marked.

16.9 Interim Analysis

Due to early closing of Part A, there will be no interim analysis in part A.

17 Sample size calculation

The number needed has been calculated in 100 per arm (i.e., 600 overall, including assumed 10% of dropouts, for the 6 planned initial arms).

17.1 Sample size calculation with multiplicity adjustment:

Sample size calculation has been carried out considering adjustment for multiplicity within each cohort.

When the sample size is 90 per group (without dropouts, i.e., 100 including 10% dropouts), two-sided simultaneous 95% confidence intervals (with Bonferroni adjustment for 2 simultaneous confidence intervals in a cohort) for a proportion using the large sample normal approximation will extend no more than ±12% (percentage points) from the observed proportion. E.g., if the observed proportion is 50% (where the confidence interval is widest), the confidence interval ranges from about 38% to 62%.

PLEASE NOTE:

Part A of the present trial in which individuals received a 3rd vaccination (first booster) was closed to further recruitment as of January 13, 2022. With the massive roll-out of booster campaigns throughout Europe, Part A was abandoned because of a poor recruitment rate. The sample size calculation provided above is the sample size calculation taken from the initial protocol for part A (protocol version V04_0). But the actual sample size will be determined by number of patients included in part A before its closure on January 13, 2022.

Which precision can be achieved when calculating confidence intervals for the primary endpoint based on smaller sample sizes we refer to additional calculations provided in section 12 of the main part of the SAP.