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Heterologous primary and booster COVID-19 vaccination

Evidence based regulatory considerations

1. Introduction

During the spring of 2021[1], a number of European Union (EU) Member States (MSs) started to apply a strategy of heterologous primary vaccination, with at least 11 EU MSs vaccinating with a first dose of Vaxzevria followed by a second dose of Comirnaty due to uncertainties related to the risk of Thrombosis with thrombocytopenia syndrome (TTS) following Vaxzevria. This decision by public health authorities was based on preliminary results from independently conducted observational studies and clinical trials. Moreover, a heterologous vaccination strategy has historically been applied for other vaccines [2]. Nevertheless this measure raised questions linked to the quality and amount of data underpinning the decision.

The focus has subsequently expanded to understand the benefits and risks of a heterologous boosting regimens, i.e. in which the primary vaccination series consisting of 1 or 2 doses (one dose was studied and authorised only for the Janssen COVID-19 vaccine) is followed by a third dose given at least 3 to 6 months later with a different severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine.

Understanding the efficacy, durability of protection and safety of heterologous primary and booster regimens against SARS-CoV-2 is important to support alternative vaccination strategies and programmatic flexibility amid supply delays and safety concerns that have slowed vaccination campaigns.

Moreover, emerging evidence indicates that heterologous primary or booster vaccination could improve the immune response as compared to homologous vaccination, at least with certain combinations. Results support consideration of strategies for maximising the level of protection that can be obtained.

This document includes a summary and appraisal of the available evidence from clinical studies and real world evidence to support the use of heterologous vaccination against Coronavirus Disease (COVID-19) within primary series and/or for booster doses. The option of heterologous vaccination is not yet reflected in the product information of authorised vaccines. Marketing authorisation holders are encouraged to submit variations to add details about such use to the product information.

[1] https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-overview-vaccination-strategies-deployment-plans-6-may-2021.pdf
[2] Shan Lu. Heterologous prime boost vaccination. *Curr Opin Immunol* 2009; **21:** 346-51

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Although the review did not look at vaccines not yet licensed in the EU, research into heterologous combinations of these will be taken into account in future if these are licensed.

The screening of the scientific literature was not based on formal systematic criteria and the cut-off date for data collection was 3 December 2021. Studies with less than 10 participants were not included. Vaxzevria, Comirnaty, SpikeVax, Janssen COVID-19 vaccine, Curevac's mRNA vaccine, Novavax' vaccine, Valneva's vaccine and CoronaVac are called respectively AZ, BNT, Moderna, JJ, CVn, NVX, VLA and CV throughout this document.

This document was adopted by the EMA Pandemic Task Force for COVID-19 (COVID-ETF) on 14 December 2021.

2. Summary of evidence on heterologous primary vaccination

Main studies on immunogenicity and safety

The **Com-COV clinical trial** is a multi-centre, participant-masked, randomised non-inferiority trial where all four prime-boost permutations of the AZ and BNT vaccines both at 28-day and 84-day prime-boost intervals were compared in 830 individuals 50-69 years of age.

In the initial manuscript published by **Shaw R. et al.**[3] only reactogenicity data were presented consisting of self-reported solicited local and systemic symptoms collected in the 7 days after each dose in participants randomised to receive vaccines at 28-day intervals.

Overall greater systemic reactogenicity was seen after the heterologous boost dose than their homologous boost counterparts. Among the most common adverse events (AEs), feverishness was reported by 24% more people (95% Confidence Interval (CI) 13–35%) after the AZ+BNT schedule, and by 20% more people after the BNT+AZ schedule as compared to their homologous counterparts, and this was accompanied by increased paracetamol usage (about 20% increase). Similar increases were observed for chills, fatigue, headache, joint pain, malaise, and muscle ache, and were mostly seen in the 48 hours post immunisation but all reactogenicity symptoms were short lived and no AEs led to hospitalizations. Of note, the reported analyses are descriptive, as the study was not powered for reactogenicity. No concerns arose from the limited haematology and biochemistry data available, which showed similar profiles between heterologous and homologous vaccine schedules.

In a second publication from the Com-COV trial, **Liu et al.** [4] reported the safety and immunogenicity of AZ and BNT vaccine schedules in the 28-day boost study groups. The primary endpoint was the geometric mean ratio (GMR) of serum SARS-CoV-2 anti-spike Immunoglobulin G (IgG) concentration at 28 days after boost, when comparing AZ/BNT with AZ/AZ, and BNT/AZ with BNT/BNT in participants who were seronegative for SARS-CoV-2 infection at baseline. Immunological secondary outcomes included anti-spike binding IgG concentration, cellular responses measured by Interferon gamma (IFNγ) ELISpot in peripheral blood, and pseudotype virus neutralisation titres at days 0, 28, and 56. Of the 830 subjects enrolled, a small subset (n=100) were enrolled into an immunology cohort, who had four additional blood tests to evaluate antibody kinetics further; these participants were randomly assigned (1:1:1:1) to the four schedules (28-day interval only).

[3] Robert H Shaw et al. Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data *The Lancet*, May 2021 https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)01115-6/fulltext
[4] Liu et al., Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8346248/

Binding IgGs were measured by a standardized ELISA, and reported as ELISA Laboratory Unit (ELU)/ml; 50% neutralising antibody titre (NT_{50}) were measured by a qualified pseudotype virus neutralisation assay (PNA), using a vesicular stomatitis virus backbone, and by a live SARS-CoV-2 virus microneutralization assay (for a limited set of samples). The results were not presented in the assigned unit as per WHO International Standards (IS) (i.e. international units (IU)/ml for neutralizing antibodies and binding antibody units (BAU)/ml for IgG) but the correlation factors for the conversion were provided (for the assays: PNA, receptor binding domain (RBD) ELISA and Pre-Spike IgG ELISA).

The heterologous schedules were considered non-inferior to the approved homologous schedules if the lower limit of the one-sided 97·5% CI of the GMR of serum SARS-CoV-2 anti-spike IgG concentration at 28 days after boost was greater than 0·63. The study was powered to 90% at a one-sided 2,5% significance level.

The results showed that the geometric mean concentration (GMC) of SARS-CoV-2 anti-spike IgG at day 28 post-boost for recipients of both heterologous schedules (AZ+BNT and BNT+AZ) were higher than the GMC of the homologous AZ vaccine schedule, however only the AZ+BNT demonstrated non-inferiority to the homologous AZ+AZ schedule (12,906 ELU/mL vs. 1392 ELU/mL, with a GMR of 9·2 (one-sided 97·5% CI 7·5 to ∞). BNT/AZ (7133 ELU/mL) failed to meet NI against the homologous schedule (BNT/BNT, 14080 ELU/mL).

Exploratory analyses on T cell responses showed that their geometric mean at 28 days post boost was highest when subjects received the heterologous schedule AZ+ BNT.

Regarding safety within 28 days post-boost, no significant difference was observed between the vaccine schedules in the proportion of participants with at least one adverse event. The total number of AEs was higher for the BNT/BNT and BNT/AZ schedules (81 and 90 respectively vs. 74 and 71 for the AZ/AZ and AZ/BNT). However the proportion of AEs of grade 3 severity was highest for the AZ/BNT schedule (8.1% AZ/AZ and 11.3% AZ/BNT vs. 1.2% BNT/BNT and 7.8% BNT/AZ).

Based on unpublished data, when administering the second dose with a 12 weeks interval, less reactogenicity was reported for AZ/BNT combo as compared to the same schedule with 28 days interval. Regarding immunogenicity, the prolonged schedule increased immunogenicity further for AZ/BNT combo as compared to the homologous AZ/AZ schedule, while BNT/BNT was still superior to BNT/AZ, although the latter difference was much less pronounced than after the 4 weeks interval.

Supportive studies on immunogenicity and safety

EICOV and COVIM[5] are prospective cohort studies conducted by the Berlin Institute of Health and Charité to assess reactogenicity and immunogenicity of heterologous immunisation in 380 health care workers (median age 35 years) who were offered AZ prime followed by BNT boost 10-12 weeks later (COVIM study) compared with homologous AZ vaccination with a similar interval, or homologous BNT/BNT with a 3-week interval (EICOV study). Blood samples for detection of SARS-CoV-2-specific antibodies and T-cell responses were collected immediately before the first vaccination, and 3–4 weeks after the first and second vaccination. Binding IgG were measured using a commercially available microarray-based immunoassay containing both Spike (S) and nucleocapsid (NC) protein to discriminate between vaccinated and infected people. The functional neutralisation capacity was measured by a commercially available RBD-ACE2 binding inhibition assay as well as a previously published SARS-CoV-2 pseudovirus neutralisation assay (pNT) against the Alpha (B.1.1.7) and Beta

[5] David Hillus et al. (Charité) Safety, reactogenicity, and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1-nCoV19 and BNT162b2: a prospective cohort study. https://pubmed.ncbi.nlm.nih.gov/34391547/

(B.1.351) variants of concern (VOCs). Maturation of IgG avidity and S-specific T cell responses were measured by a modified and standard commercially available assay, respectively.

Participants were asked to fill in electronic questionnaires on reactogenicity, adverse events, medications, and medical visits on days 1, 3, 5, and 7 after the first and second vaccination, and to self-report any systemic symptoms and intake of pain medication through an electronic questionnaire every 2 weeks after that.

No major differences in reactogenicity among the prime-boost regimens were seen. Local reactions were frequently observed for all vaccines. Systemic reactions, including severe reactions, were most frequent after prime immunisation with AZ, whereas reactogenicity of homologous BNT, homologous AZ, and heterologous AZ–BNT were similar, with slightly decreased systemic reactions after heterologous AZ+BNT and homologous AZ schedules, in contrast with the initial reporting from the Com-COV study.

Antibody levels 3 weeks post second dose were comparable among the homologous and heterologous vaccinations as well as neutralizing antibody titres (but the heterologous regimen was numerically higher, albeit non-significant), as measured by the surrogate virus neutralization assay. Significantly higher antibody avidity and T cell IFNy release were measured in the group receiving the heterologous combo. Anti-S1-IgG avidity, S1-reactive T-cells, and neutralising capacity against two variants of concern were significantly increased 3 weeks after heterologous AZ+BNT boost compared with homologous BNT and AZ boost vaccination.

The main limitations of the study design are the lack of randomisation and masking, the small sample size (especially the homologous AZ group) and different size of the various groups, and the different interval between schedules. Additionally, conversion factors to the WHO international standard were not reported for the serological assays, although these were said to be performed according to manufacturer instructions.

CombiVacS[6] is a phase 2, open label, adaptive, randomized, controlled, multicentre clinical trial conducted in Spain on 676 adults under 60 years old (mean age 44 years), vaccinated with a single dose of AZ between 8 and 12 weeks before screening, and no history of SARS-CoV-2 infection. Participants were randomly assigned (2:1) to receive BNT (0.3 mL, single intramuscular injection) or observation. The safety primary outcome was 7-day reactogenicity: reactions were predominantly mild (68.3%) or moderate (29.9%), and consisted more frequently of injection site pain (88·2%), induration (35.5%), headache (44.4%) and myalgia (43.3%); no serious adverse events were reported. The efficacy primary outcome included 14-day anti-spike IgG response (measured by commercially available immunoassays covering SARS-CoV-2 trimeric spike protein and RBD, and reported as BAU/ml), antibodies functionality and cellular immune responses (assessed using a pseudovirus neutralization assay and IFN-γ immunoassay, respectively). Following the mRNA dose, geometric mean titres (GMTs) of IgG-RBD increased from 71.46 BAU/mL (95% CI 59.84-85.33) at baseline to 7756.68 (7371.53; 8161.96) ($p < 0,0001$). IgG against trimeric spike-protein increased from 98.4 [85.69–112.99] to 3684.87 [3429.87–3958.83]. 100% participants exhibited neutralizing antibodies (Nabs) 14 days after BNT administration, in comparison to 34.1% at enrolment. A 4-fold increase in cellular immune response was also observed.

The main limitations of the study include the absence of a control group completing the homologous AZ scheme, which was due to the AZ suspension in Spain, the small sample size and the short period of observation, which may lead to underestimating the safety of the heterologous boost.

[6] Alberto M Borobia et al. Reactogenicity and Immunogenicity of BNT162b2 in Subjects Having Received a First Dose of ChAdOx1s: Initial Results of a Randomised, Adaptive, Phase 2 Trial (CombiVacS). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8233007/pdf/main.pdf

Tina Schmidt et al.[7] enrolled in a prospective cohort study in Germany 216 individuals (mean age 40, 44, 48 years across 3 groups) after having received a homologous regimen (AZ or one of the mRNA-vaccines) or a heterologous regimen (AZ priming dose followed by a mRNA vaccine). At 14 days post-dose 2, the heterologous regimen induced significantly higher spike-specific IgG, neutralizing antibodies (both measured by a commercially available ELISA and RBD-ACE2 binding inhibition assay) and spike-specific CD4 T-cells as compared to the homologous vector boost, and all these measurements were higher or comparable in magnitude to the homologous mRNA regimens. Spike-specific CD8 T-cell levels after heterologous vaccination were significantly higher than after both homologous regimens. Cytokine expression profiling showed subtle differences among regimens in terms of predominance of IFNγ, TNFα and IL-2. Regarding reactogenicity, both recipients of the homologous vector-regimen and the heterologous vector/mRNA-combination were most affected by the priming vector-vaccination, whereas heterologous boosting was well tolerated and comparable to homologous mRNA-boosting.

Study limitations included: lack of direct comparison of immunity in the same individuals after the first vaccine dose, because data after primary vaccination were available for only a subset of the mRNA/mRNA group and the majority of vector-primed individuals were enrolled after primary vaccination; most mRNA vaccine recipients received BNT; the homologous AZ vaccine group was slightly older; small sample size.

Groß et al.[8] conducted in Ulm, Germany, a cohort study of 26 individuals aged 25-46 years that received an AZ prime followed by a BNT boost after an 8-week interval to investigate reactogenicity, antibody responses and T cell reactivity. Self-reported solicited symptoms after AZ prime were in line with previous reports and less severe after the BNT boost. To quantify antibody responses, IgG and IgM were measured as units per ml (U/ml) that correlates with the WHO standard unit for the SARS-CoV-2 binding antibody units per ml (BAU/ml). Neutralization activity was measured by a SARS-CoV-2 surrogate virus ACE2 neutralization test and SARS-CoV-2 variant specific spike pseudovirus neutralization assay. Antibody titres increased significantly over time resulting in strong neutralization titres 2 weeks after the BNT boost. Neutralizing activity against the VOC B.1.1.7 was 3.9-fold higher than in individuals receiving homologous BNT vaccination, only 2-fold reduced for variant of concern B.1.351, and similar for variant B.1.617. In addition, CD4+ and CD8+ T cells reacted to SARS-CoV-2 spike peptide stimulus 2 weeks after the full vaccination. The main limitations are the small sample size and the lack of direct comparison between different vaccination regimens.

Barros-Martins et al.[9] analysed the efficacy of the heterologous prime-boost vaccination schedule in the Hannover Medical School's COVID-19 Contact Study cohort of healthcare professionals (HCPs) and monitored responses to homologous and heterologous prime-boost COVID-19 vaccine treatment schedules in 129 AZ-primed HCPs, of whom 32 chose homologous boosting and 55 chose heterologous boosting. A group of 46 BNT/BNT vaccinated HCPs were included for comparison. Serology was performed according to manufacturer's instruction, while neutralization activity, as measured by a modified surrogate virus neutralization test, was correlated to a previously developed vesicular stomatitis virus based pseudotyped virus neutralization test.

[7] Tina Schmidt et al. Immunogenicity and reactogenicity of a heterologous COVID-19 prime-boost vaccination compared with homologous vaccine regimens. https://www.nature.com/articles/s41591-021-01464-w.pdf

[8] Rüdiger Groß et al., Heterologous ChAdOx1 nCoV-19 and BNT162b2 prime-boost vaccination elicits potent neutralizing antibody responses and T cell reactivity. (preprint) https://www.medrxiv.org/content/10.1101/2021.05.30.21257971v2

[9] Barros-Martins J. et al., Immune responses against SARS-CoV-2 variants after heterologous and homologous ChAdOx1 nCoV-19/BNT162b2 vaccination. https://www.nature.com/articles/s41591-021-01449-9

Heterologous AZ/ BNT vaccination led to a significant 11.5 fold increase for anti-S IgG (p<0.0001), which was within the range of fully BNT/BNT vaccinated individuals, compared to a 2.9-fold increase after homologous AZ vaccination (P<0.0001). Overall higher titres of IgA, higher frequencies of CD4+ and CD8+ T cell responses directed against spike protein epitopes, and higher production of IFN-γ upon re-stimulation were seen after the BNT booster. BNT booster induced significantly higher frequencies of spike-specific CD4+ and CD8+ T cells and high titres of NAbs against the B.1.1.7, B.1.351 and P.1 variants of concern of SARS-COV-2.

BNT/BNT-vaccinated and AZ/BNT-vaccinated individuals developed neutralizing antibodies to similar degrees 2–3 weeks after booster vaccination.

The main limitations of this study include: the setup did not allow for randomization of the participants, and it is thus not possible to completely exclude confounding factors; a cohort of people immunized with BNT/AZ was not available; the data were obtained in mostly healthy and relatively young HCPs, thus cannot be generalised to elderly people or to specific patient groups; the neutralizing activity was not tested against the Delta variant, and data on safety and reactogenicity after vaccination could not be collected.

Wanlapakorn et al. [10] in this prospective cohort study compared the reactogenicity and immunogenicity of the heterologous adenoviral vector vaccine (AZ) and the inactivated vaccine (CV) regimen (AZ+ CV and CV +AZ, N=48 and N=46 resp.) in healthy Thai adults vs. the homologous regimen (CV + CV and AZ+AZ, N=180, 90 subjects each group). Immunizations with CV as prime dose were given at 28-day interval, while the ones with AZ as prime dose were administered at 10-12 weeks intervals. RBD-specific antibody responses and neutralizing activities against wild-type and variants of concern after two-dose vaccination were higher in the heterologous CV-AZ and homologous AZ-AZ groups compared to the CV-CV and AZ-CV groups. When comparing post boost anti-RBD IgG, CV-AZ combo was similar to the AZ-AZ schedule, however the antibodies neutralizing activity against the WT strain was significantly higher in CV-AZ combo compared to the AZ-AZ vaccination (p < 0.001). Of note, the spike-specific IgA response was detected only in the CV -AZ group after two doses of vaccination. The total IFN-y response was detected in both heterologous groups after the two-dose vaccination. These results suggest that heterologous vaccination with CV followed by AZ vaccine could be used as alternative vaccination strategy to homologous AZ-AZ, if necessary.

Main studies on vaccine effectiveness

Nordstrom et al. (2021a)[11] performed a retrospective cohort analysis using the Swedish nationwide registries to assess the waning of the effectiveness of 2-dose vaccines over time by age, sex and vaccines received, including an heterologous vaccine administration, against symptomatic infection or against a composite endpoint of severe disease (defined as inpatient hospitalisation with COVID-19, or all-cause mortality within 30 days after confirmed infection). Symptomatic infection was defined on the basis that in Sweden people with any symptoms of COVID-19 are asked to take a test; infections were ascertained by PCR or in 4·8% of the instances by sequencing. Thus, asymptomatic disease is likely to represent a minority of cases collected in the register. Infection data were gathered until 4 October 2021 and hospitalisation or mortality data were collected till 28 September 2021. Vaccinated individuals with at least 1 dose of any vaccine in Sweden until 26 May 2021 were matched by birth year and sex with unvaccinated individuals. The cohort comprised 842,974 pairs (N=1,684,958), comprised of individuals vaccinated with 2 doses of AZ, Moderna, or BNT, and matched unvaccinated

[10] Wanlapakorn et al., Safety and immunogenicity of heterologous and homologous inactivated and adenoviral-vectored COVID-19 vaccines in healthy adults. https://www.medrxiv.org/content/10.1101/2021.11.04.21265908v3
[11] Nordstrom et al., Effectiveness of Covid-19 vaccination against risk of symptomatic infection, hospitalization, and death up to 9 months: a Swedish total-population cohort study.

individuals. A total of 51,766 individuals received heterologous AZ + any mRNA vaccination. A Cox regression model was adjusted for age and date of second dose, sex, education, Swedish nationality and eight comorbidities.

Vaccine effectiveness (VE) against symptomatic infection after 2 doses of any vaccine waned progressively over time and was influenced significantly by type of vaccine, age, sex and all diagnoses at baseline. With respect to vaccine type, VE was lower and waned faster for homologous AZ vaccine (-19% 95% CI (-97-28), from day 121), followed by BNT (from 92% at day 15-30 to 47% at day 121-180 to 23% (95% CI, -2-41) from day 211 and onwards) and by Moderna (59% after more than 180 days). From day 120 and onwards, VE of heterologous AZ-any mRNA [66% (41-80)] remained higher and waned slower than for two doses of AZ (-19%). Overall, vaccine effectiveness was lower and waned faster among men and older individuals. Vaccine effectiveness (any vaccine) against severe disease (hospitalisation or death) waned from 89% at day 15-30 (95% CI, 83-93) to 74% (95% CI, 47-87) by day 121-180, to 42% (95% CI, -35-75) from day 181 and onwards, with sensitivity analyses showing notable waning among men, older frail individuals, and individuals with comorbidities. In another sensitivity analysis, in which individuals >80 years old were excluded, in the remaining cohort the effectiveness was 80% (95% CI, 41-93) from day 181 and onwards.

The study has some limitations: a follow-up beyond 180 days was not available for some vaccine regimens and for BNT effectiveness may be heavily influenced by the higher age groups. Health care workers were not accounted for and the interval between the two vaccine doses is not mentioned.

In Finland, **Poukka et al.**[12] conducted a nation-wide register-based cohort study to estimate the effectiveness of Covid-19 vaccines among ~430,000 health care workers 16–69 years old. The study outcomes were lab-confirmed SARS-CoV-2 infection and COVID-19 related hospitalisation measured as hazard ratio (vaccinated vs. unvaccinated based on Cox regression) adjusted for age, sex, presence of medical conditions and residence in the most affected districts. The follow-up period was split in two periods based on the time of emergence of the variant Delta in Finland. The study population received the following vaccination regimens: mRNA/mRNA (74%, 315,413), AZ/AZ (3%, 14,760) and heterologous regimens (7%, 30,548). Heterologous vaccine regimens included AZ+mRNA for the main analysis. The remaining population was either unvaccinated (10%) or received only one dose of vaccine. Brand-specific VE was only calculated for mRNA vaccine regimens both homologous and heterologous (BNT+Moderna or Moderna+BNT) excluding people vaccinated with AZ vaccine.

At 14-90 days after the second dose, VE against infection was 82% (95% CI 79-85%) for mRNA, 89% (73-95%) for AZ and 80% (72-86%) for heterologous vaccine series. At 91-180 days after the second dose VE was 62% (55-68%) for mRNA, 63% (-166-95%) for AZ and 62% (30-79%) for heterologous vaccine series. VE against hospitalisation was 88% (for AZ homologous regimen) or >95% (for heterologous AZ-mRNA) during the first ten months of the vaccination campaign. No major difference in effectiveness was observed in the pre-Delta vs. the post-Delta period, indicating that lower VE may be linked to waning, and between the two brands of mRNA vaccines. In conclusion the study reported comparable protection after vaccination with mRNA and heterologous vaccine regimens, with waning against infection becoming of relevance 3 to 6 months after 2nd dose and sustained high effectiveness against severe outcomes beyond 6 months from 2nd dose.

The study is overall well designed and robust because it is based on a large high quality registry. The main limitations include longer follow-up of individuals at higher risk of infection, which may underestimate effectiveness, and longer intervals between doses for those vaccinated later vs. those vaccinated first, which affects the duration of follow up and thus may underestimate effectiveness.

[12] Poukka et al., Cohort study of Covid-19 vaccine effectiveness among healthcare workers in Finland, December 2020 - October 2021 https://www.medrxiv.org/content/10.1101/2021.11.03.21265791v2

In another study from **Nordstrom et al. (2021b)**[13] the same methodology and analytical techniques were used to assess the effectiveness against symptomatic SARS-CoV-2 infection occurring >14 days after primary series, cases of COVID-19 hospitalisation, and the risk of adverse events such as thrombocytopenia and thromboembolic events in patients receiving homologous AZ-AZ vaccine schedule (N=430,100) or heterologous vaccine schedules AZ-BNT (N=94,569) and AZ-Moderna (N=16,402). Symptomatic infection was measured until August 23, 2021, and hospitalisation for COVID-19 until July 30, 2021.

Vaccinated individuals were older and with more comorbidities than the unvaccinated ones and those receiving the AZ-BNT combination were younger than those receiving a AZ-AZ combination. After a mean follow-up time of 76 days (1-183), the adjusted VE for symptomatic infection in comparison to the unvaccinated was 67% (95% CI 59-73) for AZ-BNT and 79% (62-88) for AZ-Moderna. In comparison, VE for homologous vaccination was 50% (41-58) for AZ-AZ, 78% (78-79) for BNT-BNT and 87% (84-88) for Moderna-Moderna. Only for the homologous AZ-AZ regimen, higher age was associated with lower estimated effectiveness. No estimates of VE on hospitalisation could be measured given that only 19 cases of hospitalisation were observed (16 of which in unvaccinated individuals). No significant difference between groups was observed after adjustment for age as regards the thrombocytopenia or thromboembolic events risk.

The same limitation as in the Nordstrom et al. (2021a) study are present, i.e. no information is available about the intervals between the two doses. Sequencing was made in 4.8% of infected individuals, but the authors state that the Delta variant was predominant during the study period.

Skowronski et al.[14] used a test-negative designs in a study conducted among community-dwelling adults ≥18-years-old in British Columbia (BC) and Quebec, Canada, to assess vaccine effectiveness of two doses of homologous or heterologous SARS-CoV-2 vaccines against RT-PCR confirmed SARS-CoV-2 infection and against hospitalisation occurring on or ≤30 days after specimen collection, including variants of concern (Alpha, Gamma or Delta). Controls included all specimens that were RT-PCR-negative for SARS-CoV-2 and met inclusion/exclusion criteria. The study period was between May 30 and October 2, 2021, which is said to coincide with an infection peak mid-April (Alpha variant in Quebec and Alpha/Gamma co-domination in BC), low infection levels in early-June and start of the fourth pandemic wave in late July/early August (Delta variant). The study included a population of about 4 million adults in BC and 7 million adults in Quebec and used linkage of hospital, vaccination and testing databases. The multivariate logistic regression was adjusted for age (18-49;50-69;70-79;≥80), sex, epidemiological week and region (five subregions in both Quebec and BC). The vaccination schedules included mainly (>90%) two mRNA doses, 3% AZ and 5% mixed mRNA-AZ with AZ mainly (>99%) as first dose.

VE against SARS-CoV-2 RT-PCR confirmed infection was 90% if one dose was any mRNA vaccine and 70% when both doses were AZ, with no different outcome among the mRNA homologous or mixed series (all around 90% effectiveness). VE for both infections and hospitalisations was longer with 7-8 weeks interval versus 3-4 week interval. The authors conclude that two doses of any mRNA and/or AZ vaccines gave robust protection against hospitalisation, with no sign of decline by 5-7 months post-vaccination, and that findings support the use of mixed schedules and longer intervals between doses.

Strengths of this study are the use of large community setting databases in Canada and the sound statistical analysis. Limitations are inherent to the length of the study period with changes in the

[13] Nordstrom et al. Effectiveness of heterologous ChAdOx1 nCoV-19 and mRNA prime-boost vaccination against symptomatic Covid-19 infection in Sweden: A nationwide cohort study
[14] Skowronski et al. Two-dose SARS-CoV-2 vaccine effectiveness with mixed schedules and extended dosing intervals: test-negative design studies from British Columbia and Quebec, Canada (medrxiv.org)

testing behaviours and of the characteristics of the unvaccinated population over time, lack of adjustment for important covariates and the fact that higher risk groups were given more quickly a second dose, which might triggered lower VE associated with shorter time intervals.

Prieto-Alhambra et al.[15] performed a cohort study based on linked routinely collected health data including electronic medical records, vaccination data and laboratory tests in Catalonia to study the comparative effectiveness and safety of homologous two-dose AZ and heterologous AZ-BNT vaccination. Spanish authorities allowed citizens aged <60 years previously vaccinated with a first dose of AZ to choose between AZ and BNT for their second dose, which were chosen by 89.3% (AZ-AZ) and10.7% AZ-BNT), of 167,235 eligible individuals. A total of 14,325 people in the heterologous group were exactly matched to 14,325 in the homologous group based on age (average age 42.2), sex (62.5% of female), general practice centre and day of the second vaccination (+/- 2 days). The resulting cohorts were comparable in terms of all observed demographics, comorbidity, medicine use, area of residence, and socio-economic status. Study participants received their second doses between 27th April 2021 and 8th October 2021 and were followed-up from 1st June to 11th October 2021 (follow-up time of 1-147 days after the 2nd dose). Outcomes were SARS-CoV-2 infection defined by earliest positive RT-PCR or lateral flow test (LFT) regardless of symptoms or clinical diagnosis, venous thromboembolism (VTE), VTE with thrombocytopenia, and myopericarditis within 21 days after the 2nd dose. The analysis used Cox regression analysis and confounding assessment included age, sex, area of residence, rurality and socioeconomic status, number of RT-PCR or LFT performed, pre-existing comorbidities and long-term medicine use.

The incidence rate of COVID-19 infection was 0.13/1,000 person-years and 0.21/1,000 person-years for the heterologous and homologous vaccination respectively, with a HR of 0.61 [0.52- 0.71], favouring heterologous vaccination. The two groups had similar testing rates and safety outcomes and most of the infections occurred when Delta variant was predominant. No cases of myopericarditis were observed. Sensitivity and negative control outcome analyses confirmed these findings. There were no deaths in either group and no hospitalisation in the heterologous group vs. 4 cases in the homologous group.

Important strengths of this study is the direct comparison made between the homologous and heterologous vaccination schedules based on exact matching, which led to groups comparable as for socio-demographic and comorbidity variables and the large amount of information available on covariates in the linked databases. Weaknesses are that the choice of the 2nd vaccine was left to each person, with possible differences related to health behaviours, the limited number of cases of severe COVID-19 infection (e.g. hospitalisation) for which a longer observation would have been needed, and a study population of <60 years with results not generalisable to the whole population.

A study from Denmark by **Gram et al.**[16] provided evidence of effectiveness of the heterologous AZ+BNT regimen against SARS-CoV-2 infection of 88% (95% (CI): 83; 92) 14 days after the second dose and onwards, compared with unvaccinated individuals. This was a nationwide retrospective population-based cohort study (more than 5 million people corresponding to 97.6% of the total Danish population) based on the nationwide linked Danish registries to estimate VE against SARS-CoV-2 infection (defined as a laboratory confirmed RT-PCR SARS-CoV-2 positive test), all-cause and COVID-19 related hospitalisation and death of one dose of the AZ vaccine and the AZ-any mRNA vaccine schedule, compared with unvaccinated individuals. The outcomes of interest were SARS-CoV-2 infection, defined as a RT-PCR confirmed SARS-CoV-2 infection, and all-cause or COVID-19 related

[15] Prieto-Alhambra et al. Comparative effectiveness and safety of homologous two-dose ChAdOx1 versus heterologous vaccination with ChAdOx1 and BNT162b2: a cohort analysis | Research Square
[16] Gram MA et al., Vaccine effectiveness when combining the ChAdOx1 vaccine as the first dose with an mRNA COVID-19 vaccine as the second dose. https://www.medrxiv.org/content/10.1101/2021.07.26.21261130v1.full.pdf

hospitalisation and death. The study period was 7th February to 23rd June 2021, during which only a small proportion of cases due to the Delta variant was observed. Of 144,360 persons who received the AZ vaccine as the first dose, 88,050 (61%) and 48,501 (33.6%) received the BNT mRNA and the Moderna vaccine as the second dose, respectively. The median age was 45-46 years when the 1st or 2nd dose were administered, respectively, and 25.6% had a comorbidity. A Cox regression model adjusted for sex, heritage, comorbidity, age and time intervals after vaccination was used to obtain VE estimates.

In comparison to the unvaccinated population, the adjusted VE against infection ranged from 29% to 44% between 14 to 83 days after one dose of AZ and was not different to the null value after this period. The AZ-any mRNA vaccine schedule had an adjusted VE against infection of 66% (95% CI: 59; 72) at 0-13 days and of 88% (95% CI 83-92) from 14 days after the 2nd dose. An adjusted VE of 93% (95% CI: 80; 98) against hospitalisation due to COVID-19 was observed from 14 days after the first AZ dose until receiving a 2nd dose of a mRNA vaccine. No COVID-related death or hospitalisation was observed after the mixed schedule during the study period so no VE estimates could be calculated.

The strength of the study is the use of high-quality registers relevant to the whole Danish population with access to a large amount of data allowing adjustment for a large number of covariates. A limitation is the low representation of the delta variant in the study population based on the study period. The authors state they could not eliminate difference according to health-seeking behaviours and residual confounding may persist due to the dichotomous categorisation of covariates.

Martinez-Baz et al.[17] used a prospective enhanced epidemiological surveillance of COVID-19 to assess the product-specific COVID-19 VE in preventing infection and hospitalisation in a prospective dynamic cohort of adults (≥ 18 years old) who were close contacts of COVID-19 cases from April to August 2021 in Navarre, Spain. In this system, individuals receiving a 1st dose of AZ could choose between homologous vaccination and heterologous vaccination with BNT. Data were analysed with a Cox regression model adjusted for age group, sex, chronic conditions, contact setting month and COVID-19 vaccination status of index case.

In 119 people receiving the combination AZ-BNT (with median time since the last dose of 41 (interquartile range (IQR) 32-55) days), adjusted VE in comparison to unvaccinated individuals was 86% (95% CI 70, 93) against all SARS-CoV-2 infections (including asymptomatic and symptomatic) and 91% (95% CI 71, 97) against symptomatic SARS-CoV-2 infections. No COVID-19 hospitalisation was observed. As compared to the homologous regimens AZ/AZ or JJ (one dose), the relative VE of AZ/BNT within 90 days since the last dose was 69% (95% CI: 33-85) and 69% (95% CI: 34-86), respectively.

An important limitation of these results is that, whilst the majority of sequenced unvaccinated cases belonged to alpha variant, almost all sequenced heterologous vaccinated cases belonged to delta variant and this is not adjusted for in the analysis. However, if there is a difference in vaccine effectiveness between the two variants, this would lead to underestimate vaccine effectiveness and may not affect the comparison between different vaccination schedules. The precision of the estimates is also affected by the small number of subjects with heterologous vaccination schedule (n=119).

Supportive studies on vaccine effectiveness

Pozzetto et al.[18] performed a cohort study to compare the risk of SARS-CoV-2 infection following heterologous AZ-BNT or homologous BNT-BNT vaccination schedules, and to understand the

[17] Martinez-Baz et al. Eurosurveillance | Product-specific COVID-19 vaccine effectiveness against secondary infection in close contacts, Navarre, Spain, April to August 2021.
[18] Pozzetto et al. Immunogenicity and efficacy of heterologous ChadOx1/BNT162b2 vaccination (nature.com)

immunological basis of a difference between the two vaccination schedules. The outcomes measured in the study were documented SARS-CoV-2 infection, immunoglobulins against SARS-CoV-2 (IgG and IgA against spike protein and receptor binding domain), the virus neutralization test (plaque reduction neutralization test, pseudo-neutralization test) and the B- and T-cell function (analysis of RBD-specific memory B-cells and spike-specific T cells). The probability of SARS-CoV-2 infection was measured in 13,121 healthcare workers at the University hospital of Lyon who were vaccinated with AZ-BNT or BNT-BNT, starting January 2021, and followed up until 15 August 2021 (the declaration of infection was compulsory for staff to obtain daily allowance without loss of salary during the imposed quarantine). Of these, 60 subjects not infected before vaccination and without comorbidity were included in the immunological study after informed consent, including 29 heterologous vaccinated and 31 homologous vaccinated.

In a logistic regression analysis adjusted for age, there was a significantly lower probability of infection with the heterologous regimen than the homologous BNT/BNT regimen (0.40% vs 0.76%; p=0.04). After the second dose there was no difference in the level of IgG, but IgA levels were lower for the heterologous regimen. After the second dose neutralizing activity was significantly higher for the heterologous regimen compared to homologous regimen. The heterologous regimen was not associated with a significant reduction in neutralizing activity for the Alpha, Gamma and Delta strains compared to the Wuhan strain, in contrast to the homologous regimen, which was associated with reduced neutralising activity for the Gamma and Delta strains. After the second dose, the frequency of RBD-binding memory B-cells was higher in patients vaccinated with the heterologous regimen compared to patients vaccinated with the homologous regimen, but the T-cell response was similar for the two regimens.

A limitation of the analysis of the probability of infections in this study is obviously the logistic regression not taking into account of differences in follow-up time between the groups. However, since the authors have followed patients vaccinated in a naturalistic study, and have capped the follow-up on the same date (15 August), they may have erroneously stated they have analysed infection rate using logistic regression when they presumably would have analysed infection rate using Cox regression or other survival methods. The immunological results and risk of infection could also have been influenced by the longer time between the 1st and 2nd vaccination in patients vaccinated with the heterologous regimen compared to patients vaccinated with the homologous regimen (12 weeks vs 4 weeks). It has been shown for both vaccines that long injection time intervals (12 weeks or more) provide higher binding and neutralizing antibody titres than shorter intervals (less than 6 weeks).

Powell et al.[19] performed a cohort study (published on 15 July 2021) to compare the reactogenicity of heterologous prime-boost COVID-19 vaccination following the UK extended schedule of up to 12 weeks between doses. They identified 1,313 adults 18-75 years vaccinated either with AZ-BNT (43.6% of study population), BNT-AZ (12.7%), AZ-AZ (35.1%) or BNT- BNT (8.6%) between 29 March 2021 and 01 June 2021 through the NIMS database. An online survey was performed via text messages in order to collect details on gender, age, ethnicity, occupation, vaccination status, COVID-19 related symptoms in the past year, any post vaccination symptoms following each dose with timelines, graded by the individuals from 0 (no symptoms) to 4 (emergency department or hospital admission required).

Previously-uninfected individuals who received heterologous prime-boost schedules were 2.4 times (27.8% vs. 11.6%) more likely to report severe reactogenicity after their second dose than those receiving homologous schedules. These findings were irrespective of the reason for receiving a heterologous schedule. Reactogenicity rates were higher in younger adults, women and after the first

[19] Eurosurveillance | Real-world data shows increased reactogenicity in adults after heterologous compared to homologous prime-boost COVID-19 vaccination, March–June 2021, England

dose of AZ in any schedule. Those experiencing severe reactions after their first dose, irrespective of the vaccine type, were more than twice as likely to experience a severe reaction after the second dose compared to those reporting a no or a mild to-moderate reaction after their first dose (29.7% vs. 13.3%).

The results of this survey are difficult to interpret due to the low response rate (from 36,779 patients, only 1,313 responded to the questionnaire), lack a clarity on many aspects of the study design (e.g. no details on how they identified vaccinees in the NIMS, different timelines and possibly different inclusion criteria for the recruitment of vaccinees with homogenous and heterologous vaccination, possible recall bias and no account taken for the vaccinees' health status or differences between sub-groups), and lack of clarity in the presentation of the results and the statistical analyses performed.

Vallée et al.[20] used primary data collection of healthcare workers of the Foch Hospital, France, to assess the immunogenicity of BNT administered as second dose in healthcare workers primed with AZ. In this study, Foch hospital healthcare workers were invited for a survey and serology test in June and July 2021. Participants received either two vaccinations for BNT (n=67) or an initial dose of AZ followed by BNT (n=130). The survey collected age, gender, type and date of first dose, type and date of second dose. Serum SARS-CoV-1 IgG antibody level was measured 30 to 60 days after second vaccination.

The univariate analysis found significantly higher antibody levels in participants with two BNT doses as compared to AZ followed by BNT, but no significant difference was observed after adjustment for time duration between the first and second vaccinations. A negative correlation between antibody levels and time duration between second dose and serology test was observed for BNT-BNT which remained significant after adjustment for all covariates, but not for AZ-BNT.

This study has several limitations which restrict its validity and generalisability: a possibility of self-selection bias as patients were invited to participate and the sequence of vaccination was not given at random, a small sample size (197), participants were young healthcare professionals unlikely to be representative of the general population, there could be significant unmeasured confounding, and the time between first and second vaccination is significantly different between groups.

2.1. Conclusions on heterologous primary vaccination

- Strength of evidence: the more robust evidence among immunogenicity and safety trials was generated by the Com-COV trial and more is expected from other ongoing trials that include the homologous regimen as comparator arm. The evidence generated by observational studies is considered supportive and provides valuable additional evidence supporting the consistency of the findings.
- In terms of safety in clinical trials, reactogenicity of heterologous vaccination appears overall similar with respect to homologous regimens. However, specifically in the Com-COV study it was more pronounced in the heterologous arm (up to 24 folds in the preliminary report from the controlled Com-COV trial (N=820, >50YOA)), with fever being the most commonly reported event. Of note, this difference in reactogenicity decreased by extending the vaccination interval from 4 to 12 weeks especially for the AZ/BNT combination. The observational study by Powell et al also shows a 26% increase in severe reactogenicity following the heterologous vs. the homologous booster, however the study has several limitations that hamper the interpretation of the results.

[20] Vallée et al. An Immunogenicity Report for the Comparison between Heterologous and Homologous Prime-Boost Schedules with ChAdOx1-S and BNT162b2 Vaccines

Currently, very limited data are available from two observational studies conducted in Germany (Schmidt et al and Gross et al) for a total of nearly 600 individuals. These indicate similar reactogenicity profiles between homologous and heterologous regimens when a direct comparison is available, or the emerging profile is in line with the expectations based on existing knowledge with the authorised vaccines. The highest reactogenicity is reported after the adenoviral vaccine priming. The uncontrolled CombiVacS trial (N=676, <60YOA) reports that AEs after heterologous vaccination are mostly mild or moderate and are related to local reactions plus headache and myalgia. With respect to infrequently occurring adverse reactions, there is insufficient data to draw conclusions.

- In terms of immunogenicity findings from trials, the COMCOV study's results at 28 days interval demonstrated non-inferiority of the heterologous AZ/BNT regimen vs. AZ/AZ in terms of day 28 post boost GMR of SARS-CoV-2 anti-spike IgG concentration (PP analysis), with superiority demonstrated as secondary endpoint since the lower limit of the two-sided 95% CI around the GMR (MITT analysis) was greater than one (9·3 (95% CI 7·7 to 11·4)). BNT/AZ antibody titres were inferior to BNT/BNT induced titres. The disparity in immunogenicity between the homologous and the heterologous schedules is reduced by prolonging vaccination intervals between doses, based on unpublished data. The ELISA used to measure binding IgGs was standardised and the PNA to quantify antibody neutralizing activity was qualified, which add additional strength to this randomized trial. T cell responses were also found to be higher.

  Although it was published after the cut-off date and therefore could not be considered in detail, results from Stuart et al[21] (Com-COV2 study) seem to be aligned with the conclusions in this report.

  Immunogenicity appears robust after heterologous regimen (AZ/BNT) with more than 100 fold increase in IgG as compared to the first dose, 100% of people with Nabs and >4fold increase in CMI, based on the uncontrolled CombiVacS trial.

  The observational cohorts studies indicate that binding and neutralising antibodies and antigen-specific T-cell are significantly more pronounced after heterologous AZ/BNT than after homologous AZ/AZ vaccination, and higher or comparable in magnitude to the homologous mRNA regimens, with a predominant Th-1 biased cytokine profile. Overall the difference in immunogenicity between the heterologous regimen and the homologous mRNA regimen is less obvious, however all studies in which immunogenicity against VOCs was tested indicate that the heterologous vaccination may be able to induce an expanded breadth of cross-reactivity both in terms of humoral and cell mediated immunity. Another observational immunogenicity study showed that heterologous prime-boost vaccination strategy leads to improved immunogenicity and acceptable safety also with inactivated vaccines such as CV (CV+AZ induces significantly higher neutralising antibody titres than AZ+AZ).

- Regarding vaccine effectiveness studies, 7 of the identified studies provided an adequate level of evidence supporting the effectiveness of the heterologous vaccination against symptomatic infection. Regarding effectiveness against SARS-CoV-2 symptomatic infection, one of the Nordstrom study[13] indicates that the heterologous AZ+ any mRNA regimen affords a higher protection as compared to the homologous AZ regimen, but similar or slightly lower as compared to the homologous mRNA regimen, especially Moderna. In addition, the results from the other Swedish study (Nordstrom et al[11]) on durability of protection indicate that waning of effectiveness against symptomatic infection is higher and faster for AZ/AZ among the

[21] Immunogenicity, safety, and reactogenicity of heterologous COVID-19 primary vaccination incorporating mRNA, viral-vector, and protein-adjuvant vaccines in the UK (Com-COV2): a single-blind, randomised, phase 2, non-inferiority trial - The Lancet

homologous regimens (-19% AZ-AZ vs. 66% of AZ-BNT at 4 months post-dosing and onwards).

Other studies indicate comparable findings (e.g. AZ-BNT 62% VE against symptomatic infection at 3-6 months post-dosing, Poukka et al), supporting the use of mixed schedules. These studies produced evidence supporting: longer intervals between doses improve effectiveness, with no sign of decline in protection against hospitalisation by 5-7 months post-vaccination (Skowronski et al) or beyond 6 months after any primary series (both homologous and heterologous vector-mRNA, Poukka et al), and equivalent effectiveness among mRNA vaccines homologous and heterologous combinations including one does of mRNA (Skowronski et al); no myocarditis cases in a population of more than 30,000 well-matched individuals in the robust Spanish Study (Prieto-Alhambra et al); expanded neutralizing activity by the heterologous regimen against the Alpha, Gamma and Delta strains compared to the homologous regimen, and higher frequency of RBD-binding memory B-cells (Pozzetto et al).

- The main limitation observed so far in the immunogenicity and safety observational studies is the lack of a comparator arm/cohort in some of the studies, lack of randomisation and the limited sample size. In addition, individuals at risk and elderly may be underrepresented. Regarding effectiveness studies, the main limitations includes the variable interval between doses and type of circulating variant not accounted for, lack of sufficient DNA sequencing, small sample size or non-representativeness of the population.
- Not every possible combination has been extensively tested so far, e.g. there is limited data on interchangeability of mRNA vaccines.
- The main uncertainty in the risk profile is represented by the lack of knowledge on rare and very rare AEs that could potentially be associated with the heterologous regimen. Observational studies could provide additional evidence with respect to occurrence of (very) rare adverse events. A higher reactogenicity of the heterologous regimen is not a consistent finding among studies .
- The main uncertainty in terms of benefits of the heterologous regimen is estimating the magnitude of the efficacy of the heterologous regimen with respect to the homologous regimen based on immunogenicity. However, vaccine effectiveness studies are now becoming available, such as Nordstrom et al and Skowronski et al, with clinically relevant endpoints to indicate that an increase in immune response translates into increased protection. Indeed the overall evidence evaluated in this review show a similar or increased (up to ~20% based on some studies) effectiveness against symptomatic infection of an heterologous regimen where the first dose is AZ and second mRNA vs. homologous AZ regimen, and comparable effectiveness vs. the homologous mRNA schedule.

2.2. Recommendations on heterologous primary vaccination

- The currently available evidence consistently points towards an acceptable tolerability and enhanced immune responses with the sequential heterologous regimen of vector vaccine / mRNA vaccine vs. the homologous vector vaccine regimen.
- Some studies have reported higher reactogenicity (e.g. pain, fever, headache, fatigue) of heterologous vaccination but results are not consistent. With respect to infrequently occurring adverse reactions, there is insufficient data to draw conclusions.
- Regarding immunogenicity, studies are consistent in showing the heterologous regimen is able to induce significantly increased immune responses, including improved memory B cells,

compared with a homologous viral vector regimen. A slight increase in humoral immune responses with respect to homologous mRNA vaccination is sometimes seen, but not consistently, overall supporting a similar antibody response.

- The increased immunogenicity appears consistent with the increased vaccine effectiveness against SARS-CoV-2 symptomatic infection of the heterologous vector-mRNA regimen as compared to homologous vector immunisation based on several good quality observational studies.
- Preliminary but consistent evidence indicates that the heterologous regimen is able to induce an expanded breadth of immune responses, with improved humoral and cell mediated cross-reactivity against various variants of concerns, which would translate into improved effectiveness based on the studies seen so far.
- Overall the data presented support the use of mixed vector/mRNA schedules. Based on the evidence seen so far and on existing clinical knowledge, giving a second dose of mRNA vaccine to previous recipients of a single dose of vector vaccines is a vaccination strategy that is beneficial from an immunological perspective with a positive impact on the achieved level of protection from infection and disease. There is less evidence about heterologous mRNA vaccination regimens, but enough to indicate that such an approach could be used as well when flexibility or acceleration in the vaccination campaigns is needed. Safety data after such heterologous mRNA regimens are currently under investigation to determine if there is an increased risk of myocarditis.
- Giving an adenoviral vector vaccine as second dose after a mRNA vaccine might be considered if there is a problem with availability of mRNA vaccines, but based on the limited data available it may be less advantageous from an immunological point of view than the opposite sequence.
- Long term protection data after heterologous or homologous primary vaccination is limited, but a few studies suggest a decline in protection against symptomatic infection from 6 months after heterologous vaccination. Some of these studies also show that waning of effectiveness is greater and faster for Vaxzevria homologous regimen than other regimens and that waning is overall faster among older frail individuals, and individuals with comorbidities.
- More research is needed to investigate use of heterologous regimens in immunosuppressed individuals.

3. Summary of evidence on heterologous booster vaccination

Main studies on immunogenicity and safety

Atmar et al.[22] published preliminary results of a phase 1/ 2 open label clinical trial conducted with 458 individuals in the US enrolled in two age groups, 18-55 years and ≥ 56 years, who received one of three Emergency Use Authorisation (EUA) COVID-19 vaccines at least 12 weeks prior to enrolment to receive a booster injection with one of three vaccines (Moderna 100µg, JJ 5×10^{10} virus particles, or BNT 30µg), with nine combinations in total (50 individuals per group and 25 per age stratum). 3 of the 4 vaccines for which the EMA granted conditional marketing authorisation were included in the trial, except AZ.

The booster dose was administered at an interval after second dose ranging between 12 and 20 weeks for most treatment groups, whilst for 3 groups ranged from 12 to 30 or 41 weeks and for one group

[22] Atmar RL, et al. (2021). Heterologous SARS-CoV-2 Booster Vaccinations – Preliminary Report. medRxiv. https://www.medrxiv.org/content/10.1101/2021.10.10.21264827v2

ranged from 11 to 23 weeks (intervals were shortest for those who were boosted with Moderna). Of note, for Moderna the trial sponsor used double the authorised dose for booster vaccination, which is 50µg. The primary outcomes were safety, reactogenicity and humoral immunogenicity at day 1 (pre-vaccination), 15 and 29 post boost (the latter data are not available for some groups for binding and neutralising antibodies so none of these data have been considered here).

Serum binding antibody levels against wild type virus were measured with the MSD 384-well Custom Serology Assay Electrochemiluminescence Immunoassay (4-plex ECLIA, currently under validation), and with a previously described 10-plex ECLIA (not qualified nor validated) against variants of concerns (B.1.617.2 or Delta and B.1.351 or Beta) . To assess the magnitude, kinetics, duration, and breadth of SARS-CoV-2 neutralizing antibody responses, a fully validated assay in an environment that operates in compliance with Good Clinical Laboratory Practice was used. Assays were performed on all samples with pseudotyped lentiviruses presenting SARS-CoV-2 Spike D614G. A subset of samples were also tested for variants, however the assay is in the process of being validated for Beta, but has not been validated for Delta. Part of the IgG serum antibody responses to wildtype strain and IU_{50} neutralizing antibody titres to pseudovirus D614G were bridged to international standards and reported as BAU/mL and international units ID_{50}/mL (IU_{50}/mL), respectively.

Local and systemic AEs were reported within 7 days after administration of the boosters. Injections site AEs (mostly mild) and systemic malaise, myalgia and headache were commonly reported (2 severe injection site reactions were reported for JJ and Moderna). Unsolicited AEs through 28 days following vaccination also were collected. The percentage of boosted participants reporting unsolicited AEs, of any severity grade, that were deemed related to the study product ranges between 12% and 16% across groups. Most participants ranked the reported related AEs as Grade 2 severity. There were four related Grade 3 AEs (vomiting, fatigue, insomnia) reported after JJ and Moderna. All serious adverse events (only 2 reported, unrelated to study treatment), new onset chronic medical conditions (none reported), adverse events of special interest (one severe vomiting leading to a medically attended visit after JJ boost), or related medically attended adverse events (MAAEs) are collected for the duration of the 12-month study and are reported in the publication until study day 29. There were no patterns of reaction frequency for solicited or unsolicited AEs by primary EUA vaccine received or age group. Overall reactogenicity and adverse events did not differ between homologous and heterologous boosters and no safety concerns were identified.

All booster vaccines were immunogenic irrespective of the primary regimen. The fold increases from baseline in both binding and neutralizing antibody titres were similar or greater after heterologous boosters compared to homologous boosters. The combinations inducing less GMTs (both binding and neutralising antibodies) are the homologous JJ and the BNT/BNT+JJ combinations (IgGs: GMTs 326 and 1900 BAU/ml respectively). All other combinations reached GMTs higher than 3000 BAU/ml. The geometric mean fold rises in binding antibodies titres at Day 15 ranged from 4.6 to 56 and were highest for those who received BNT (33 fold) and Moderna (56 fold) boost after JJ as primary series. The combinations inducing the lowest fold rise as well as the lowest percentage of people with 4-fold rise over baseline at day 15 post boost (both for binding and neutralising antibodies) are the homologous JJ and the heterologous Moderna/Moderna+JJ combinations (GMT fold rise 4.2 and 6.2 and % with 4-fold rise 50% and 61% respectively for neutralising antibodies).

Serum neutralization (IU_{50}/mL) levels prior to booster vaccination were approximately 3-and 10-fold lower for BNT and JJ recipients, respectively, compared to recipients of Moderna, irrespective of interval between EUA vaccination or booster vaccination administered. Looking at the effect of the booster, the Day 15 post-boost neutralization titres ranged from 676.1-901.8 IU_{50}/ml for participants boosted with Moderna, 31.2-382.2 IU_{50}/ml for those boosted with JJ, and 341.3-677.9 IU_{50}/mL for those boosted with BNT. Looking at the effect of the primary series, Day 15 post-boost neutralizing

GMTs were highest in Moderna-primed participants, followed by BNT and JJ, regardless of the booster vaccine administered. The lowest neutralising GMTs were recorded for the JJ homologous booster (31.4 IU_{50}/ml (95% CI 22.3-44.3)) and for the BNT/BNT+JJ combination (216.4 IU_{50}/ml (157.8-296.9)). Similar findings are reported based on IU_{80}/mL neutralization levels.

Overall JJ was also able to boost all primary regimen, but homologous JJ booster achieved a level of neutralising 7-12-fold lower compared to when JJ boosted subjects who received a mRNA vaccine priming regimen. Similar trends are seen for the binding antibodies.

All groups, with the exception of the homologous JJ prime-boost group, achieved post-boost neutralizing geometric mean IU_{50}/mL levels of >100 against wild type virus, which in a previous study[23] correlated with 90.7% vaccine efficacy for preventing symptomatic disease.

With respect to variants of concern, all boosters induced binding antibodies against the Delta variant in all participants irrespective of age. By comparison, binding antibody titres were 34-45% lower than wild type pre-booster and 15-36% lower than wild type virus post-booster (no difference by age).

Neutralization data against the Delta and Beta variants are still not fully available at the time of writing, but neutralising activity increased substantially following booster vaccination among people already evaluated to levels suggestive of protection against severe disease and death by Delta based on knowledge with the parent strain.

The main limitations of this well-conducted study include the non-randomised open label design, low number of samples per group especially from a safety perspective, study not designed to compare among boosters (e.g. different intervals for booster administration), safety follow-up not sufficient for identifying late adverse events after booster, and double dose for the Moderna booster vs. the recommended SmPC dose. Cell mediated immunity and duration of response are not known.

The **COV-BOOST[24]** phase 2 randomised controlled double-blind trial was conducted in 18 sites in the UK, in a mixture of community and secondary care settings, on 2883 (randomised) individuals aged 30 years and older, half of whom had received 2 priming doses of AZ at least 70 days before and half 2 priming doses of BNT at least 84 days before. Half of the study population was aged 70 years and older but only mild to moderate well-controlled comorbidities were permitted.

The study sites were split into three groups (A, B, and C), in which participants were randomly assigned to receive one of seven COVID-19 booster vaccines (plus 3 of them were administered also as half doses) or control for a total of 26 treatment combinations. Each group (A, B or C) had their own control and included both primary series populations (AZ/AZ and BNT/BNT). Group A received as a booster either NVX, a half dose of NVX, AZ, or quadrivalent meningococcal conjugate vaccine (MenACWY) as control. Group B received as a booster either BNT, VLA, a half dose of VLA, JJ or MenACWY. Group C received either Moderna, CVn, a half dose of BNT, or MenACWY. The randomisation schedules were stratified by study site, age (above and below 70 years of age (YOA)) and subgroup (as explained below). Moderna booster was administered at a dose of 100µg, whereas all the others authorised vaccines followed the official posology (except for the half doses).

Participants were screened and vaccinated at day 0. Blood was taken for immunogenicity analyses at days 28, and will be taken at day 84, and 365. A separate subgroup of 25 individuals from each group (n=650 participants in total) were subject to additional bleedings at day 7 (to detect evidence of

[23] Gilbert et al., Immune Correlates Analysis of the mRNA-1273 COVID-19 Vaccine Efficacy Trial https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8366808/pdf/nihpp-2021.08.09.21261290v4.pdf

[24] Munro et al, Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial, https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02717-3/fulltext

previous immunological priming via rapid spike IgG responses) and day 14 (to detect the peak T-cell response).

The study participants were given diary cards to record solicited adverse events on day 7, unsolicited adverse events on day 28, and medically attended adverse events on day 84. The adverse events recorded in the cards or collected during the study visits included adverse events of special interest and serious adverse events.

SARS-CoV-2 anti-spike IgG concentrations were measured by ELISA (reported as ELISA laboratory units [ELU]/mL), neutralising antibodies by SARS-CoV-2 pseudotype virus neutralisation assays (Nexelis, Laval, QC, Canada), and cellular responses by T-cell assays (Oxford Immunotec Abingdon, UK).

Safety/reactogenicity and immunogenicity were coprimary outcomes. The reactogenicity outcomes are mentioned above; the immunogenicity outcome is anti-spike protein IgG at day 28 in each group reported as GMC and 95% CI; the GMR and 99% CI (to account for multiple comparison) between each group and the corresponding control group was also reported. Secondary endpoints included neutralising antibody titres against wild-type Wuhan strain, and pseudovirus neutralisation and T-cell response (measured by ELISpot) against wild-type virus and Delta variant.

The study was designed to have 90% power to compare the GMC of anti-spike IgG between each COVID-19 vaccine group with the control group within each of the three groups (A and C [three comparisons], and B [four comparisons]) and populations (AZ/AZ and BNT/BNT). 111 participants/ group were required as sample size to detect an established minimum clinically important difference of 1.75-times difference in GMC and to account for seropositives at baseline and lost to follow up.

Reactogenicity seemed higher after a vector booster (both AZ and JJ) post BNT priming and after Moderna and Curevac boosters regardless of the vaccine used for priming. Overall the JJ booster seems more reactogenic than the AZ booster. The type of local and systemic reactions were similar among groups, with fatigue, headache and pain the most common reactions reported.

In the AZ/AZ primed group, all boosters induced significantly higher IgGs at 28 days post-boost compared with their corresponding controls as the upper limit of the 99% CI of the GMRs was higher than the pre-established minimum clinically important difference of 1.75 (the lowest GMR was 1.8 (99% CI 1.5–2.3) for VLA). In the BNT/BNT primed group, a similar result was seen, however VLA did not reach the pre-established limit (GMR 1.3 with 99% CI 1.07-1.62). GMRs for neutralising antibodies against wild type were consistent with those for IgGs.

Looking at binding antibodies at day 28 post boost, the most immunogenic booster in individuals primed with AZ/AZ were Moderna and BNT. Moderna reached the highest levels of antibodies and GMRs, but the dose is also higher (100µg Moderna vs. 30µg for BNT). The GMCs reached by BNT heterologous booster (AZ/AZ/BNT, GMC 20,517, 95% CI (17,718-23,757)) were numerically lower than the GMCs of the BNT homologous regimen (BNT/BNT/BNT, GMC 27,242 95% CI (24,148-30,731)). NVX half, JJ and CVn induced comparable levels of antibodies in AZ primed individuals, whilst NVX performed better (up to 2x higher), and all performed 2/3 times better than AZ homologous booster. The most immunogenic booster in individuals primed with BNT was Moderna followed closely by BNT homologous booster. AZ, NVX and JJ were able to boost to a similar level of GMCs (but reached half GMRs compared to Moderna). JJ seemed to boost equally well individuals primed with AZ or BNT but higher GMCs were reached with BNT/BNT/JJ than with AZ/AZ/JJ. JJ seems to be able to boost better than AZ irrespective of the primary series. The best booster overall appears to be Moderna and the best combination BNT/BNT/Moderna, however it is important to note that Moderna was also given at the highest dose compared to other mRNA vaccines.

All the boosters, except for AZ and VLA in AZ/AZ-primed participants and for VLA in the BNT-primed participants, induced higher cellular responses compared to the control as measured by T-cell ELISpot.

Regarding neutralising antibodies, at day 28 post boost JJ reached comparable GMTs after BNT primary series (GMT 1441, 95% CI (1188-1749)) to the homologous BNT booster (BNT/BNT/BNT, GMT 1789, 95% CI (1520-2107)), whilst the difference remained when boosting after AZ primary series (GMT JJ 563 vs 1621 for BNT). Against the Delta variant, a heterologous JJ boost after BNT primary series is similar to the BNT homologous 3 dose series (GMT 418 vs. 392 respectively).

Similar GMRs for neutralising antibodies were observed between the Delta variants and the wild type virus for all vaccine combinations, when comparing with control groups, which suggests that no booster vaccine induces better cross-protective immunity than others based on these data.

After the booster dose, IgG and cellular responses were similar between age groups. However a larger difference among age groups was seen when boosting with NVX (GMC 8400 in >70YOA vs. 5800 >70YOA) in AZ primed people, but the difference was smaller in individuals primed with BNT.

Interestingly, in the BNT/BNT study arm, already at day 7 post boost, BNT and Moderna were able to induce anti-spike IgG binding antibodies at same level than at day 28 post boost, while JJ and AZ levels were lower at day 7 and peaked at day 28, indicating a different kinetic of immune responses for the two types of platform technologies. This was not seen when AZ was administered as primary series vaccination, where JJ booster effect was considerably lower than BNT at day 7 as well as at day 28. This latter data suggest that the type of vaccine used for priming is crucial in determining the quantity and quality of booster responses, however it has to be noted that the outcomes are descriptive. AZ, NVX, and Curevac boosters, similarly to JJ, also induced a further increase from day 7 to day 28 following either primary series albeit to a lower level, with the exception of the AZ homologous booster (AZ/AZ/AZ). The peak of T cellular response measured by ELISpot was observed at day 14 post-boost for each combination.

The study is overall well designed and conducted and, the methodology robust. There are some limitations related to shorter interval to dose 3 in some participants as compared to the interval between dose 1 and dose 2 of the primary series, which might have underestimated GMRs and also negatively affected the response to the booster; individuals <30YOA are not included; racial diversity was very limited; the half dose for Moderna could not be tested; T cell results are known to be affected by interlaboratory variability which hampers comparability with other studies; Alfa and Beta variants were not tested and the neutralisation assay for the variants was not validated.

Li et al.[25] conducted a randomized, controlled, observer-blinded proof of concept trial of heterologous prime-boost immunization with CV and Convidecia (AD5-nCOV, Cansino) in healthy adults 18-59 years of age. 300 participants, who were primed with one or two doses of CV (administered 1/3 months or 3/6 months before enrolment, respectively), were randomly assigned at a 1:1 ratio to receive a booster dose of Convidecia or CV. Primary endpoints were the occurrence of adverse reactions within 28 days after vaccination and GMTs of neutralising antibodies measured at 14 and 28 days after the booster vaccination by a microneutralization assay with a wild-type SARS-CoV-2 virus strain ($PRNT_{50}$). Solicited and unsolicited adverse events were collected via diaries in the 1 days after vaccination and serious adverse events were documented for the whole study duration. The WHO international standard for anti-SARS-CoV-2 immunoglobulin was used as reference for calibration and harmonization of the serological assays. The peak of neutralizing antibody at day 14 after heterologous boost reached 197.4 (95% CI 167.7, 232.4) (vs. 33 (28.3, 39.8) homologous CV boost) and 54.4 (37.9, 78) GMTs

[25] Li et al., Heterologous prime-boost immunization with CoronaVac and Convidecia. https://www.medrxiv.org/content/10.1101/2021.09.03.21263062v1

(vs. 12.8 (9.3, 17.5) for homologous CV boost) for the three-dose and two-dose regimens respectively. The difference remained substantial at day 28 post-boost. Slightly higher incidences of injection-site reactions were found following the heterologous vaccination of Convidecia vs. the homologous booster with CV but no severe safety issues were reported. No serious adverse event was seen in any cohort of the study.

The Erasmus Medical Centre is the sponsor of the **SWITCH Trial**[26], a single-(participant)-blinded, multi-centre, randomized controlled trial which enrolled 434 healthy healthcare workers aged 18 to 65 vaccinated with a single dose of JJ. The subjects were randomized 3 months post primary series to receive a homologous JJ booster, a heterologous booster with BNT or Moderna vaccine or no boost. The prime-boost interval was 84 days (-7 days / +21 days). The vaccines were administered according to the Summary of Product Characteristics, except for Moderna which was administered at a dose of 100µg. The key objectives of this trial are to measure reactogenicity and humoral immune responses (binding, neutralising antibodies and virus-specific T cells) against SARS-CoV-2 after inoculation with a single-dose JJ vaccine compared to a homologous JJ 2-dose vaccination regimen and to compare a homologous vaccination regimen (JJ/JJ vaccine) with a heterologous vaccination regimen (JJ /BNT + JJ /Moderna vaccine).

The primary outcome was the determination of binding antibodies by a quantitative standardized high throughput assay 28 days after booster comparing the 3 study groups mentioned above. Results indicate that the SARS-CoV-2-specific binding antibodies in the heterologous mRNA-based booster vaccinations were significantly higher than homologous JJ boosting regimen ($p<0.001$), with the Moderna combination performing better than BTN combination ($p=0.01$). Boosting induced 100% response rate with mRNA vaccines and 97% response rate with JJ (3 out of 106 subjects did not have detectable antibodies). Neutralizing antibodies and T cell responses were highest among participants receiving any mRNA heterologous boost. All but one subject in the JJ boost group who had no neutralising antibodies before booster showed detectable levels after booster.

No safety or reactogenicity concerns were identified. Moderna boosting was associated with higher reactogenicity, but no adverse event required hospitalization.

Supportive studies on immunogenicity and safety

In a pilot prospective cohort study conducted in Lebanon, **Moghnieh et al**[27] tested the safety and immunogenicity of a BNT booster dose in 50 COVID-19-naïve individuals aged 29-75 years who had received 3 months earlier two doses of the Sinopharm BBIBP-CorV vaccine, , which is a whole virion β-propiolactone inactivated aluminium adjuvanted vaccine. This group was compared with a group of 50 COVID-naïve individuals matched by age and gender who received only 2 doses of BNT as primary vaccination. Heterologous booster vaccination with BNT was found to be safe and well tolerated with pain and tenderness at site of injection the most common AEs reported and to induce significantly higher anti-spike IgG geometric mean titres compared to the homologous BNT primary immunization [(8,040 BAU/mL, 95% confidence interval (CI), 4,612-14,016) vs. (1,384 BAU/mL, 95%CI, 1,063-1,801), respectively, ($P < 0.0001$)]. The GMTs after BNT heterologous booster were similar to the GMTs induced by one dose of BNT in individuals who recovered from COVID-19, which were assessed

[26] Sablerolles RSG et al., Immunogenicity and reactogenicity of booster vaccinations after Ad26.COV2.S priming. https://www.medrxiv.org/content/10.1101/2021.10.18.21264979v1

[27] Moghnieh R et al., Immunogenicity and reactogenicity of BNT162b2 booster in BBIBP-CorV-vaccinated individuals compared with homologous BNT162b2 vaccination: Results of a pilot prospective cohort study from Lebanon. https://pubmed.ncbi.nlm.nih.gov/34656379/

in a third group (N=25). Study limitations include lack of randomisation, small sample size, variable boosting interval and lack of neutralising antibody measurement.

A randomised control trial by **Ka Pun Mok et al**[28] investigated a third dose of CV or BNT after two doses of CV. 80 subjects 34-73 years of age, who were classified as low responders after 2 doses of CV vaccine, were randomized to receive an additional dose of either BNT (n=40) or CV (n=40) approximately 4 months after. The primary outcomes included humoral immunogenicity measured by surrogate virus neutralisation test (sVNT), 50% plaque reduction neutralization tests ($PRNT_{50}$) and ELISA at one month after booster. The secondary outcome was the occurrence of adverse reactions within 7 days and 1 month after the third dose of vaccination. Significantly more participants in the BNT group reported pain and swelling at the injection site, as well as fatigue and muscle pain than subjects receiving homologous third dose. Participants who had received BNT showed significant higher levels of specific antibodies against Spike receptor binding domain (RBD) ($p<0.0001$), N-terminal domain (NTD) ($p<0.0001$) and the membrane fusion subunit S2 ($p<0.0001$) as detected by ELISA, as well as significantly higher neutralization capacity with respect to CV booster. Similar results were seen against different VOCs.

Huat NKK et al [29] evaluated Spike-specific humoral and cellular immunity in 55 JJ vaccinated individuals who were either primed with JJ only (n=13), or boosted with a homologous (JJ, n=28) or heterologous (BNT, n=14) second dose, compared with the results found in individuals vaccinated with a single (n=16) or double (n=44) dose of BNT. All the analyses performed in this study were conducted in an exploratory manner and the number of samples per group was limited. The booster dose increased overall the humoral immune responses in all individuals irrespective of their first vaccination. Heterologous boost expanded the breath of humoral and cellular immunity, while homologous JJ boost did not enhance cellular immunity but simply increased the quantity of anti-Spike antibodies targeting preferentially the S1 chain of the Spike protein and not expanding to the S2 .

Keskin Au et al.[30] published immunogenicity results in 68 health care workers who received a third dose of CV (n=18) around 6 months after the primary series as compared to the subjects (n=27) who received a BNT booster ~6 months post first dose of CV. 23 non vaccinated and non-infected health care workers were used as control. A third dose of CV induced 1.7 and 1.8 fold increase in median values of IgG-S (Spike) and IgG-N (nucleocapsid) titres, respectively; BNT administration as the third dose boosted IgG-S median titres by a factor of 46.6, and IgG-N titres decreased by a factor of 6.5.

Angkasekwinai et al.[31] conducted a single-centre prospective cohort study on safety and immunogenicity data of different booster vaccine platforms (BBIBP-CorV, inactivated vaccine manufactured by Sinopharm; AZ; full dose (30 µg) of BNT; and half dose (15 µg) of BNT) administered to 352 healthy adults aged 18-60 years, who received a 2-dose primary series of CV 4 weeks apart (N= 179) or AZ vaccine 8-10 weeks apart (N= 173).

To record solicited local and systemic adverse reactions, participants self-assessed signs or symptoms in an electronic diary for 7 days after vaccination. Among the groups primed with AZ vaccine, the highest percentage of AEs were recorded in the subjects receiving BNT, followed by AZ and lastly by BBIBP-CorV. This was different when CV was given as primary series, in which case the highest

[28] Ka Pun Mok C. et al., A RCT of a third dose CoronaVac or BNT162b2 vaccine in adults with two doses of CoronaVac. https://www.medrxiv.org/content/10.1101/2021.11.02.21265843v1
[29] Huat NKK et al., Differential immunogenicity of homologous versus heterologous boost in Ad26.COV2.S vaccine recipients. https://www.medrxiv.org/content/10.1101/2021.10.14.21264981v1
[30] Keskin et al., SARS-CoV-2 specific antibody responses after third CoronaVac or BNT162b2 vaccine following two-dose CoronaVac vaccine regimen. https://pubmed.ncbi.nlm.nih.gov/34536028/
[31] Angkasekwinai N et al., The immunogenicity and safety of different COVID-19 booster vaccination following 2 CoronaVac or ChAdOx1 nCoV-19 primary series https://www.medrxiv.org/content/10.1101/2021.11.29.21266947v1.full.pdf

reactogenicity was registered in AZ booster group followed by both BNT booster groups and ultimately by BBIBP-CorV. Of note, no serious AEs were recorded.

Immunogenicity was evaluated by measuring the levels of anti-RBD IgG against the wild type virus and neutralising antibodies by a plaque reduction neutralisation test against Delta and Beta variants. Plasma was isolated pre-booster dose and 2 weeks after booster vaccination. Anti-RBD IgG levels were quantified by a specific chemiluminescent microparticle assays and results were converted to WHO international standard and reported in BAU/ml.

In the subjects immunized with AZ, the geometric mean concentration of anti-RBD IgG at 2 weeks post booster was highest when receiving 30µg BNT vaccine as booster (2,364 BAU/ml), followed by half dose of BNT (1,962 BAU/mL), AZ (246.4 BAU/mL), and lastly BBIBP-CorV (128.1 BAU/mL). A similar trend was measured in the plasma of the subjects vaccinated with CV as primary series. Regardless of the vaccine platform used as booster, the post-boost geometric mean concentration levels in the subjects immunized with 2 doses of AZ were generally lower than in the those immunized with CV. Of note, the antibody neutralization capacity against VOCs was highest in the subjects boosted with BNT vaccine either if they received AZ or CV as primary series vaccination. PRNT50 antibodies were 1.5-fold higher against Delta as compared to Beta variant for both primary series.

The major study limitations are the relatively small sample size, the difference size of booster groups, and the fact that the study was conducted open label.

In line with other studies, a booster dose of BNT is more immunogenic than a booster dose of AZ when given to individuals previously immunized with AZ as primary series.

Sabrina Tan et al.[32] conducted an immunogenicity study to compare the humoral and cellular responses of 65 individuals aged 23-84 years vaccinated with BNT as primary series and then boosted at least after 6 months with the homologous BNT (N= 24) or JJ vaccine (N=41). Antibodies in sera were measured at 2 and 4 weeks post-boost against wild-type, Beta and Delta strain.

JJ and BNT boosts increased the median of RBD-IgG titres as measured by ELISA against wild-type virus and Delta and Beta variants compared to baseline (pre boost). Anti-RBD IgG ELISA titers against all the strains were higher for BNT booster group compared to the JJ group at 2 week timepoint (JJ 11,264 vs BNT 30,730 for wild-type; JJ 10,817 vs BNT 26,398 for Delta and 5,375 vs 14,725 for the Beta strain). The ELISA antibody titres among the two groups reached comparable levels at 4 weeks post boost, although a limited number of samples was tested at this timepoint.

Pseudovirus neutralizing antibody responses were boosted by both BNT and JJ compared to post-primary series antibody levels. JJ boosting increased median neutralizing antibody titres against the wild-type, Delta, and Beta variants to 1,462, 1,009, and 899 at week 2 following the boost, respectively, and these titres further increased to 3,597, 2,198, and 1,924 at week 4 following the boost. BNT boosting increased median neutralizing antibody titres against the wild type, Delta, and Beta variants to 7,554, 2,978, and 1,865 at week 2 following the boost, respectively, and these titres slightly declined to 5,553, 1,968, and 1,576 at week 4 following the boost. Regardless of the decline, BNT-induced levels of neutralizing antibody titres remained higher at week 4 against the wild-type strain as compared to JJ-induced titres, while the median neutralizing antibody levels against the Delta and the Beta Strain were comparable between JJ and BNT. Median RBD-specific memory B cell responses were boosted similarly by BNT and JJ vaccine at week 2.

[32] Sabrina Tan et al., Ad26.COV2.S or BNT162b2 Boosting of BNT162b2 Vaccinated Individuals https://www.medrxiv.org/content/10.1101/2021.12.02.21267198v1

The main weakness of this study is the limited sample size, including fewer samples tested at week 4 (8 per group), which hampers a conclusion. The trends suggest a different kinetics of antibody responses between the 2 boosters.

Ireland G et al.[33] conducted a prospective cohort study in London UK to evaluate SARS-CoV-2 antibody responses before and after booster vaccination with BNT vaccine in healthy individuals older than 50 years of age (N=750). Before receiving the booster, subjects were immunized either with two BNT doses <30 days apart (BNT-control), with two BNT doses > 30 days apart (BNT-extended) or 2 AZ doses > 30 days apart (AZ-extended). SARS-CoV-2 spike protein antibody geometric mean titres before and 2-4 weeks after booster were compared, as measured by the commercially available Elecsys Anti-SARS-CoV-2 S total antibody assay.

GMTs were significantly increased by the booster vaccination as compared to antibody levels after primary vaccination in all three groups: 18,104 (95%CI, 13,911-23,560; n=47) in BNT-control (76.3-fold), 13,980 (11,902-16,421; n=118) in BNT-extended (15.9-fold) and 10,799 (8,510-13,704; n=43) in AZ-extended (57.2-fold) participants. Subjects in the BNT-control group had the largest post-booster increase in GMTs (76.3-fold), followed by the group receiving heterologous boost in the AZ-extended group (57.2-fold). In agreement with other publications, BNT vaccine was able to induce high antibody responses, irrespective of the vaccine used for primary immunisation. Of note, subjects immunized with BNT <30 days schedule had a longer interval (median:262 days) between primary series and booster than subjects vaccinated with either BNT or AZ >30 days schedule (both median:186 days). These results suggest that longer intervals between primary series and booster doses may favour the induction of higher antigen-specific immune responses.

Main studies on vaccine effectiveness

Andrews et al.[34] used a test-negative case control design to estimate VE of a booster dose of BNT vaccine against PCR-confirmed symptomatic disease. Vaccination status in symptomatic adults over 50 years of age with PCR-confirmed SARS-COV-2 infection was compared with the vaccination status in individuals who reported symptoms but had a negative SARS-COV-2 PCR test. The data source was NHS testing data linked to the NIMS database, which contains information on potential confounding variables. The analysis used logistic regression with the PCR test result as the dependent variable where those testing positive are cases and those testing negative controls, and was adjusted for age (5 year bands), sex, index of multiple deprivation, ethnic group, care home residence status, geographic region, period, health and social care worker status, clinical risk group status, clinically extremely vulnerable, severely immunosuppressed, and previously testing positive. Vaccine effectiveness was assessed for each primary course of vaccine with a BNT booster in 0 to 1, 2 to 6, 7 to 13, 14+ day post booster vaccine intervals. In the primary analysis, those who had received the booster were compared to individuals who had received 2 primary doses with at least 140 days prior to the onset but with no booster dose recorded. The study population included 13,569 unvaccinated people, 149,434 people who received AZ 140 days post a second dose and 84,506 people who received BNT 140 days post a second dose. Of these, 6,716 had received an AZ primary course and 17,521 received a BNT primary course.

As compared to unvaccinated individuals, the VE of a two-dose schedule (without booster) was 44.9% (38-51) for AZ and 62.5% (61.0-63.9) for BNT. A three-dose schedule with BNT as 3rd dose was

[33] Ireland G et al., Serological responses to COVID-19 booster vaccine in England https://www.medrxiv.org/content/10.1101/2021.11.22.21266692v1
[34] Andrews et al. Effectiveness of BNT162b2 (Comirnaty, Pfizer-BioNTech) COVID-19 booster vaccine against COVID-19 related symptoms in England: test negative case-control study (khub.net)

93.1% effective when the primary schedule was AZ and 94.0% effective when the primary schedule was BNT.

As compared to a two-dose-schedule of AZ, VE of the same schedule plus a BNT booster was 87.4% (84.9 to 89.4). A three-dose schedule with only BNT had a VE of 84.4% in comparison to a two-dose schedule of BNT.

These data indicate a very high protection against symptomatic disease induced by BNT booster regardless of the primary series (BNT or AZ). To understand long term protection after booster further follow up is necessary. The strength of the study is the large amount of information on potential confounding factors, the large sample size and the robust study design. A limitation is the imperfect sensitivity of the PCR testing and the uncertainty regarding the time schedule of the PCR testing in relation to the symptoms, but inclusion/exclusion criteria applied in the study probably attenuated possible misclassifications. Residual confounding may still be present but the direction of this confounding, if present, would tend to underestimate vaccine effectiveness.

In a very large **effectiveness study conducted in Chile**[35], the Chilean government reported preliminary results on the effectiveness of booster doses, based on data from two million people (out of a cohort of 11 million people) who had received two doses of CV, and a third booster dose of CV, BNT or AZ vaccines. Protection against COVID-19 increased from 56% after CV primary series to 80% after CV booster, 90% after BNT booster and 93% after AZ booster (VE measured at 14 days after the booster). Protection against hospitalization increased from 84% after primary CV series to 88%, 87%, 96% after CV, BNT and AZ respectively (14 days post-boost).

3.1. Conclusions on heterologous booster vaccination

- In general heterologous boosting is immunogenic regardless of the type of vaccine used in the primary series, with no specific safety concerns emerging to date. Especially when boosting with a mRNA vaccine, immune responses, estimated as binding and/or neutralising antibodies, are similar or higher than homologous mRNA boosting and higher than homologous vector boosting including against variants of concerns.

- On the basis of the data published by Atmar et al, all heterologous boosters studied should be able to induce an anamnestic response that leads to a significant increase in immunogenicity. All the studied combinations except the homologous JJ prime and boost regimen were able to reach high levels of neutralising and binding antibodies associated with high level of protection[24].

  Although the study by Atmar et al. was not statistically powered for making direct comparisons across vaccine regimens as primary objective, the main conclusion is that a homologous boost with JJ vaccine results in significantly lower immune responses compared with homologous mRNA boosting or heterologous boosting, with either mRNA or JJ vaccine. The sequence of priming and boosting may affect the antibody level, e.g. boosting with a mRNA vaccine after a single JJ vaccine dose produces higher antibody levels than priming with 2 doses of mRNA followed by a JJ booster (based on both binding and neutralising antibodies at day 15 post-booster). GMTs of binding and neutralising antibodies are higher after any heterologous booster than after their homologous boost counterpart except for Moderna for which the homologous booster reaches similar GMTs, albeit at a higher dose than what is currently approved. Heterologous boosting with either BNT or Moderna after any primary series was able to induce a robust antigen-specific binding and neutralizing antibodies response, and the

[35] Chile VE study https://www.minsal.cl/wp-content/uploads/2021/10/2021-10-07-EFECTIVIDAD-DOSIS-DE-REFUERZO_ENG.pdf (full study not published)

higher titres seen with Moderna should not be overinterpreted considering the higher than approved dose used (100µg for Moderna). Data at 29 days post-boost are being collected and have thus not been considered in this report. Data on long-term follow up and on cell mediated immunity are not available. Data on variants are still being collected but preliminary results are encouraging.

The COV-BOOST trial in the UK in almost 3000 individuals indicates that booster doses of seven COVID-19 vaccines, including all the vaccines authorised in Europe, are generally well tolerated and induce a substantial increase in immune responses at day 28 post-boost. In particular, mRNA vaccines provide a stronger booster effect based on binding antibodies, regardless of whether the primary course is BNT or AZ. However, JJ heterologous boost after BNT primary series (but not after AZ primary series; Moderna primary series not studied) is able to raise neutralising antibodies to levels comparable to those induced by BNT homologous series, both against the wild type as well as against the Delta variant.

Similar results are also seen in the small study by Tan et al. at the same timepoint, indicating that boosting with a vector or a mRNA vaccine after mRNA priming may induce comparable levels of neutralising antibodies at 1 month post-boost. BNT superiority to JJ in boosting is more evident when looking at neutralising antibodies at 2 weeks post-booster, which suggests that antibody kinetics of a vector vaccine are different than a mRNA vaccine and the antibodies induced by an adenoviral vector booster would peak at around 1 month post-booster, whereas the antibodies induced by a mRNA boost peak earlier post-booster in these studies.

The Dutch SWITCH trial conducted in 434 healthy healthcare workers aged 18 to 65 years showed that at day 28 post-booster SARS-CoV-2-specific binding antibodies were significantly higher in the heterologous mRNA-based booster group than those seen in the homologous JJ boosting regimen ($p<0.001$). Neutralising antibodies were also highest after a mRNA booster at the same time point. However for all the three trials mentioned, it is not possible to define whether there is an actual difference in immunogenicity between the Moderna and BNT boosters due to the different posology.

An observational study in 55 subjects reported an expanded breadth of humoral and cellular immunity after heterologous JJ/BNT vaccination vs. homologous JJ vaccination.

- Some of the other studies reported in this review showed that in subjects primed with inactivated vaccines the highest antibody increase is induced by heterologous mRNA vaccines boosters as compared to vector vaccines or inactivated vaccines.
- Effectiveness data after the various booster combinations are currently limited. Among the few available effectiveness studies, a study in the UK by Andrews et al. showed a substantial increase in protection against symptomatic disease in people aged 50 years and older after a BNT booster regardless of the primary vaccination series (mRNA or AZ). Unpublished data from a Chilean study indicates that a heterologous or homologous booster reaches a high level of protection against hospitalisation with no major differences across boosters (BNT, CV or AZ) after a primary series with the inactivated vaccine CV.
- In conclusion, data are limited overall but the trials and observational studies available provide evidence of adequate quality. The available evidence suggests that heterologous boosters are protective and safe, however the data on safety are limited, especially for the long term. With regards to the heterologous mRNA combinations the incidence of myocarditis and pericarditis should be continuously monitored. The sequence of vaccination affects the level of antibodies, in all cases. The heterologous combination vector priming/mRNA boosting is generally more

immunogenic than the opposite sequence (mRNA priming /vector boosting), however further review might be needed as some of the data at different time points are still being collected and emerging evidence suggest antibody kinetics may vary by vaccine type. Heterologous mRNA regimens appear the most immunogenic combinations, but it is not possible to conclude on the effect of the Moderna booster as Moderna was systematically used at double the authorised booster dose. The homologous vector booster strategy appears to be least immunogenic overall across studies. The heterologous regimens induce improved neutralising antibodies as compared to the homologous vector regimens also against the Delta variant.

In key studies such as Atmar et al. the booster dose was given after approximately 3 months post dose 2 therefore supporting the possibility to have a shorter interval between primary series and booster dose if needed.

3.2. Recommendations on heterologous booster vaccination

- The evidence available so far with different types of authorised vaccines indicates that a heterologous booster appears as good as or better in terms of immune responses than a homologous booster. Among the heterologous booster combinations, boosting with a mRNA after a vector primary series is more immunogenic than the reverse. In addition the safety profile of heterologous and homologous booster combinations remains comparable based on the data available.
- A heterologous booster vaccination strategy can thus be considered as an alternative strategy, e.g. to improve protection that can be achieved with some vaccines, to allow more flexibility in case of issues with vaccine acceptance, supply or vaccine availability. Data currently available support safe and effective administration of a booster dose as early as 3 months from completion of the primary vaccination should such short interval be desirable from a public health perspective and notwithstanding current recommendation to administer booster preferably after 6 months.
- Safety data provide limited but reassuring information with respect to short term reactogenicity for any booster combinations. A heterologous booster dose of viral vector vaccine or Spikevax tend to give more adverse events related to local or systemic reactogenicity. Large observational studies will provide additional evidence with respect to occurrence of rare adverse events, such as myocarditis, with either homologous of heterologous boosters.
- While it would be expected that higher immune response will translate into increased protection against infection and disease including from different VOCs, due to the lack of established correlates of protections it cannot be precisely defined at this stage to what extent such an improved immunogenicity would translate into higher effectiveness. However emerging effectiveness data show increased protection from symptomatic disease after heterologous boosting with a mRNA vaccine during spread of the Delta variant.
- Administration of booster doses, whether homologous or heterologous, needs to take into account waning of protection over time and optimal interval for an efficient immune response. At the moment there are no data in immunosuppressed individuals to support a recommendation for heterologous boosting.