








Review

## Heterologous prime–boost strategies for COVID-19 vaccines

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### Abstract

**Background/Objective:** Heterologous prime–boost doses of COVID-19 vaccines (‘mix-and-match’ approach) are being studied to test for the effectiveness of Oxford (AZD1222), Pfizer (BNT162b2), Moderna (mRNA-1273) and Novavax (NVX-CoV2373) vaccines for COVID in ‘Com-Cov2 trial’ in UK, and that of Oxford and Pfizer vaccines in ‘CombivacS trial’ in Spain. Later, other heterologous combinations of CoronaVac (DB15806), Janssen (JNJ-78436735), CanSino (AD5-nCOV) and other were also being trialled to explore their effectiveness. Previously, such a strategy was deployed for HIV, Ebola virus, malaria, tuberculosis, influenza and hepatitis B to develop the artificial acquired active immunity. The present review explores the science behind such an approach for candidate COVID-19 vaccines developed using 11 different platforms approved by the World Health Organization.

**Methods:** The candidate vaccines’ pharmaceutical parameters (e.g. platforms, number needed to vaccinate and intervals, adjuvanted status, excipients and preservatives added, efficacy and effectiveness, vaccine adverse events, and boosters), and clinical aspects must be analysed for the mix-and-match approach. **Results** prime–boost trials showed safety, effectiveness, higher systemic reactogenicity, well tolerability with improved immunogenicity, and flexibility profiles for future vaccinations, especially during acute and global shortages, compared to the homologous counterparts.

**Conclusion:** Still, large controlled trials are warranted to address challenging variants of concerns including Omicron and other, and to generalize the effectiveness of the approach in regular as well as emergency use during vaccine scarcity.

**Key words:** COVID-19, heterologous prime–boost, mix-and-match, SARS-CoV-2, vaccine, vaccination

## Introduction

Till 7 December 2021, 136 coronavirus disease 2019 (COVID-19) vaccines are in various phases of clinical trials (10 in phase 4 and 29 in phase 3), while 194 are in the pre-clinical phase.<sup>1</sup> Generally, vaccines require multiple shots, and receivers should be injected with the same type of biological preparation in the second dose as the first (known as ‘homologous prime–boost’ vaccination).<sup>2</sup> The first dose primes the body’s immune system and the second dose amplifies the immune response at an effective level with a different vaccine product than the first dose. Priming would be for the primary series where two doses use ‘mix-and-match’ strategies, and boosting would be suitable for those vaccines with the completed primary schedule but are usually done with another vaccine platform. The strategy of combining different vaccines during the prime and boost phases that target the same antigen (known as the ‘heterologous prime–boost’ or ‘mix-and-match’ strategy) has already been successfully deployed for the treatment of numerous conditions, including human immunodeficiency viruses (HIV),<sup>3,4</sup> Ebola virus disease (EVD),<sup>5</sup> malaria, tuberculosis, influenza and hepatitis B.<sup>4</sup> The World Health Organization (WHO), with support from the Strategic Advisory Group of Experts (SAGE) on Immunisation and its COVID-19 Vaccines Working Group has been evaluating evidence on the use of heterologous priming schedules. Heterologous boosting refers to administering a vaccine from a vaccine platform different from the vaccine used to complete the primary vaccine series.<sup>6</sup>

Nevertheless, the value of this strategy awaits results yet to be developed from a clinical trial for the treatment of COVID-19. In EVD vaccines, the prime shot of adenovirus vector carrying the gene for the viral protein, followed by a booster of modified vaccinia Ankara encoding the same viral gene, was adopted.<sup>7,8</sup> The same approach was adopted for the COVID-19 vaccine being developed by the City of Hope Medical Center, USA (COH04S1).<sup>1</sup> Two unique vaccine administrations targeting the same or overlapping antigens but using different delivery systems or vectors are used, intended to improve immunogenicity and efficacy compared to the same vaccine in two shots.<sup>9</sup>

The combination of DNA vaccine, encoding the spike protein (S) and S1 recombinant protein vaccine, was shown to induce high neutralizing antibody response and T-cell response in animal models.<sup>10</sup> Studies showed that the heterologous vaccine administration could overcome the adverse effects of immune response to viral vectors<sup>11</sup> and improve immunogenicity.<sup>12</sup> However, Iacobucci (2021) reported that the mix-and-match approach for Oxford and Pfizer vaccines (AZD1222 and BNT162b2, respectively) caused mild-to-moderate reactions.<sup>13</sup> Shaw *et al.* (2021), in their interim safety analysis, reported that haematology and biochemistry profiles were similar in both heterologous and homologous uses of these vaccines. Vaccine-associated events (VAEs) were of grade 2 severity or less in the heterologous scheme and without thrombocytopenia for Day 7 post-boost. However, they observed increased systemic reactivity with the boost dose of heterologous schedules, compared to the homologous, which was manageable with paracetamol.<sup>14</sup>

Generally, immunocompetent individuals develop an immune response to any infectious agents as the innate immune responses

such as cytokine and interferon secreted by the immune cells act immediately to show antiviral activity once the antigen enters the body.<sup>15,16</sup> Also, the specific immune response acts against the virus or antigen after 6–8 days of infection due to T cell (cellular response) and B cell (antibody response).<sup>15</sup> The cellular immune response components, i.e. CD8+ cytotoxic T cells, kill the virus-infected cells with the help of perforin and granzymes and slow down and stop the infection, whereas CD4+ helper T cells cause B cells activation for antigen-specific antibody production. The activated B cells then produce plasma cells (i.e. antibodies-producing cells) and memory B cells respond to the antigen instantly if the same antigen is encountered in future.<sup>15</sup>

In COVID-19, the body produces antibodies against the viral S (S1 and S2 subunit) and N protein. The neutralizing antibodies target the receptor-binding domain (RBD) present in the S1 subunit,<sup>17</sup> whereby IgM, IgG and IgA neutralizing antibodies appeared. All the antibodies against the viral protein can be detected in serum within 1–3 weeks after infection.<sup>15</sup> Specifically, seroconversion of IgM and IgG occurs at ~2 weeks post-onset of symptoms and reaches neutralizing titre in 14–20 days.<sup>18</sup> A study conducted in Italy revealed that serum anti-spike IgG antibody titre increased from 16 400 to 23 800 arbitrary units per millilitre.<sup>19</sup> Neutralizing antibody titre may remain stable for 75–180 days in the case of COVID-19.<sup>20</sup> A large cohort study also found anti-S IgG antibody titre stable over 5 months for the virus neutralization.<sup>21</sup> IgM reaches a lower level by the fifth week and almost disappears by the seventh week, whereas IgG antibodies persist for a long time.<sup>22,23</sup> Serum IgA antibody’s function in SARS-CoV-2 infection is not yet known, but secretory IgA antibody provides local mucosal immunity.<sup>24</sup> Studies noted that memory B cells specific for SARS-CoV-2 spike (S) protein were found in the individuals recovered from the infection.<sup>25,26</sup> The level of S-specific memory B cells remained stable for more than 5 months after the infection and get associated with viral neutralization, unlike antibodies.<sup>27</sup> In normal conditions, resting (RM) subset of memory B cells is prevalent but decreases during many viral infections. A recent study also found that severe COVID-19 infection-activated (AM) and tissue-like (TLM) subsets were significantly increased.<sup>28</sup>

Providing the identical vaccine in the first and subsequent shots may not always be possible, especially during pandemics such as COVID-19, due to manufacturing delays and poor supply systems. The Public Health England policy, therefore, permits the use of different vaccines under certain conditions.<sup>2</sup> Heterologous prime–boost strategy is being studied to evaluate its effectiveness against COVID-19 in the UK (called ‘Com-Cov2 trial’ to test for Oxford, Pfizer, Moderna (mRNA-1273) and Novavax (NVX-CoV2373) vaccines) in 1050 volunteers<sup>5,29</sup> and Spain (‘CombivacS trial’ to test for Oxford and Pfizer vaccines) in 663 volunteers.<sup>30</sup> Preliminary data of these trials revealed that participants in the heterologous groups experienced minor VAEs such as fever (more in the UK trial and less in the Spanish trial).<sup>30</sup> Nevertheless, other combinations of vaccines have to be trialled to identify the best combination for the treatment of COVID-19. Hence, the present review explores the strategy of heterologous prime–boost regimes as a robust means of vaccinating large numbers of people safely and efficiently in the face of challenging vaccine rollout conditions.

## Rationale for Heterologous Priming

The primary factor in heterologous priming is the lack of availability or limited and unpredictable supply of the same COVID-19 vaccine in several settings. Such interchangeability of vaccine products would allow for their flexible application. Other key reasons include investigating the use of heterologous priming concerning reactogenicity, immunogenicity and vaccine efficacy (VE). However, heterologous priming should be instituted only when evidence supporting the said parameters is available.<sup>6</sup>

## Current State of Knowledge

The available data on heterologous priming vaccine schedules have been continuously monitored, with guidance being available in some product-specific interim recommendations (such as for mRNA vaccines, i.e. BNT162b2 or mRNA-1273, and ChAdOx1-S [recombinant] vaccines to date). The WHO recommends that the same vaccine product be used for both doses of COVID-19 vaccination even during emergency use with a two-dose primary series schedule. However, no additional doses of either vaccine are recommended if different COVID-19 vaccine products are administered in the two doses. The mix-and-match schedules currently available constitute off-label use of respective vaccines and, as such, should only be used when the benefits outweigh the risks.<sup>6</sup>

Studies on the immune response after the first dose of ChAdOx1-S [recombinant] followed by an mRNA vaccine (i.e. BNT162b2 or mRNA-1273) show higher neutralizing antibody levels and higher T cell-mediated immune response compared to two doses of either of the two. The sequence of ChAdOx1-S [recombinant] for the first dose followed by the mRNA vaccine for the second dose was more immunogenic than the reverse. However, these findings should be carefully interpreted due to limited sample sizes and lack of follow-ups, about safety profiles, immunological outputs and clinical impacts. The initial results on short-term VE following a heterologous schedule were obtained from Denmark, showing an 88% (95% CI: 83–92%) effectiveness when following the said sequence. The VE result was similar to VE of two mRNA vaccine doses in a population-wide register-based study when the SARS-CoV-2 Alpha variant was dominant. More observational data on safety and effectiveness are expected.<sup>6</sup>

The candidate vaccines' pharmaceutical parameters (e.g. platforms, number needed to vaccinate and intervals, adjuvanted status, excipients and preservatives added, efficacy and effectiveness, VAEs, boosters), and clinical aspects must be analysed for the mix-and-match approach.

## Candidate Vaccines' Platforms

Till 7 December 2021, the candidate COVID-19 vaccines were reported to exploit 11 different platforms: 47 (35%) protein subunit (PS), 22 (16%) RNA, 20 (15%) non-replicating viral vectors (VVnr), 18 (13%) inactivated virus (IV), 15 (11%) DNA and 14 (9%) other platforms (6 virus-like particles (VLP), 2 replicating viral vector (VVr), 2 combined VVr and antigen-presenting cell (APC), 2 live attenuated viruses (LAV), 1 combined VVnr and APC, and 1 bacterial antigen-spore expression vector).<sup>1</sup> Out of many different subunit vaccines, only the PS platform has been

trials for COVID-19 vaccines due to safety issues. However, as adjuvants are usually required with such vaccines, there is a potential risk of VAEs from adjuvants.<sup>31</sup>

Two types of whole virus COVID-19 vaccines (i.e. live attenuated and inactivated) have been trialled for COVID-19, necessitating stringent safety checks to ensure that the attenuated viruses do not revert to wild-type.<sup>31</sup> Killed vaccines may not produce life-long immunity and require booster doses at different time intervals,<sup>32</sup> and VLPs (assembled from viral structural proteins) have adjuvant properties.<sup>33</sup> The endogenous antigen production would stimulate both humoral and cellular immune responses by a single dose.<sup>31</sup> Currently, administered mRNA vaccines developed by Pfizer and Moderna were found to produce required immunogenicity against SARS-CoV-2.<sup>34</sup> Previously, DNA vaccines were approved only for veterinary<sup>35</sup> and will be a novel approach for human use in the case of COVID.<sup>36</sup>

## Adjuvanted Candidate Vaccines

Inactivated vaccines were traditionally developed without adjuvant, but it has been used in some COVID-19 vaccines to promote immunogenicity.<sup>10</sup> Novavax is a recombinant glycoprotein vaccine adjuvanted with Matrix M; Sanofi Pasteur (VAT00002), Vaxine (NCT04453852) and Medigen Vaccine Biologics (MVC-COV1901) also used S protein with adjuvant; Clover Biopharmaceuticals (SCB-2019) and Nanogen Pharmaceutical Biotechnology (NANOCOVAX) used S protein with alum adjuvant, while Vaxart vaccine (VXA-CoV2-1) is an Ad5 adjuvanted oral vaccine.<sup>1</sup> However, the exact immunologic basis of adjuvants like aluminium salts (e.g. aluminium hydroxide, aluminium phosphate) is still unknown. Although adjuvanted aluminium salts are generally safe,<sup>37</sup> these may cause local reactions (e.g. injection site pain, inflammation, granulomas formation, abscesses, lymphadenopathy and skin ulceration) and systemic reactions (e.g. fever, headache, malaise, diarrhoea, arthralgia and myalgia).<sup>38</sup>

## Excipients and Preservatives in Candidate Vaccines

Pfizer and Moderna vaccines contain polyethylene glycol (PEG or macrogol) as the major excipients (i.e. compounds other than the active pharmaceutical ingredient (API) that are intentionally included in a drug delivery system that has been assessed for safety) that stabilize the lipid nanoparticles containing mRNA. In contrast, Oxford and Johnson & Johnson (JNJ-78436735) vaccines contain polysorbate 80 (Tween 80). These excipients are most frequently associated with allergic reactions.<sup>39</sup> The WHO draft does not indicate the presence of any preservative (e.g. thiomersal or other), antimicrobials (e.g. neomycin, polymyxin B or streptomycin) and medium of egg cultures in any candidate vaccine.<sup>1</sup> Therefore, preservative and egg culture-related VAEs (e.g. anaphylactic reactions) seem not to be in the formulation of any of the COVID-19 vaccines.

## Number Needed to Vaccinate, Intervals and Site of Inoculation

It is still under investigation how many people need vaccination against COVID-19 before population immunity is achieved

and how effective the vaccines are against the new variants of COVID-19.<sup>40</sup> Previously, it was reported that ~67% of the population need to be vaccinated for herd immunity against SARS-CoV-2.<sup>30</sup> The prime concerns about the factors that need to be in place for this target to be achieved are the requirements of scale-up of manufacturing capacities and maintenance of proper distribution channels and supply systems worldwide.<sup>31,41</sup> Based on the mathematical models, it has been projected that low- and middle-income countries would suffer from the disproportionate distributions of vaccines till 2023 due to hurdles such as cold-chain requirements, manufacturing delays and imbalanced supply channels.<sup>42</sup>

Of all candidate vaccines under various phases of clinical trials till 7 December 2021, 114 (84%) were injectable (104 (76%) intramuscular, 5 (4%) subcutaneous, and 5 (4%) intradermal), 8 (6%) intranasal, 4 (3%) oral, 1 (1%) aerosol and 1 (1%) inhaled. Eighty-three (61%) candidate vaccines required two shots (43 on Day 0–28, 33 on Day 0–21 and 7 on Day 0–14), 21 (15%) required a single shot and two (1%) required 3 jabs (on Day 0–28–56).<sup>1</sup>

## Vaccine Adverse Events

Immunogenicity of vaccines is usually worse among older adults due to immunosenescence caused by the decreased availability of T-cells and B-cells. Therefore, immune response assessment is necessary for COVID-19 vaccine development for these groups, adjuvanted vaccines being suitable for them.<sup>43</sup> The Oxford vaccine was found to have low reactogenicity and mild side effects, safe and well-tolerated among older adults.<sup>14,43,44</sup> However, few adverse events were reported with the boost dose with declining reactogenicity on advancing age, necessitating low dose for all age groups.<sup>43</sup> Both the Pfizer and Moderna vaccines have also been shown to produce mild-to-moderate reactions, with very few severe allergic reactions.<sup>34</sup> Mix-and-match approach for Oxford and Pfizer vaccines caused more mild-to-moderate reactions than the standard regimen. Fever was recorded among 37 (34%) of 110 recipients of Oxford vaccine as prime and Pfizer as boost dose, compared with 11 (10%) of 112 recipients of Oxford for both prime and boost. Fever was reported in 47 (41%) of 114 recipients following the deployment of the Pfizer vaccine as prime and Oxford as boost dose, compared with 24 (21%) of 112 recipients of Pfizer as both prime and boost. Although similar increases were also seen for other reactions such as chills, headaches and myalgia, such reactions were transient.<sup>13</sup>

## Booster Doses

Boosters for COVID-19 vaccines may be required because the first dose can only unreliably activate the body's immune system, and the second provides consistent protection against COVID-19. Immunity is better three months after the Moderna vaccine and six months after Oxford shots, but still, there is no evidence of how long immunity to COVID-19 persists after vaccination. Furthermore, since coronavirus is rapidly mutating (similar to the flu virus mutating every year), our immune cells may not recognize the mutated virus, and thus, we may need to administer a booster vaccine to tackle new strains.

**Table 1.** Details of candidate vaccines and trials<sup>1,5,29,45</sup>

Trial	Platforms	Schedule (days)	ROA	Dose	Adjuvant	Excipients <sup>a</sup>	Developers	Phase	Efficacy (%)	VAEs	Storage
Com-Cov2 (UK)	VVnr	0–28	IM	5x10 <sup>10</sup> vp (0.5 mL)	-	Polysorbate 80	AstraZeneca and University of Oxford <sup>b</sup>	4	76–100	Tenderness, pain, warmth, redness, itching, swelling	Regular fridge
	mRNA	0–21	IM	30 µg (0.3 mL)	-	PEG	Pfizer/BioNTech and Fosun Pharma <sup>b</sup>	4	95	Chills, headache, pain, tiredness, redness, swelling	-70°C
	mRNA	0–28	IM	0.10 mg mRNA(0.5 ml)	-	PEG	Moderna and NIAID	4	86	Similar to Pfizer vaccine	-20 °C
	PS	0–21	IM	5 µg SARS-CoV-2 rS + 50 µg Matrix-M1 adjuvant (0.5 ml)	Matrix M	PEG	Novavax	3	96.4–100	None reported	Regular fridge

IM: intramuscular; NIAID: National Institute of Allergy and Infectious Diseases; PEG: polyethylene glycol; PS: protein subunit; ROA: route of administration; VAE: vaccine adverse events; VP: viral particles; VVnr: non-replicating viral vectors; WFI: water for injection.

<sup>a</sup>Important excipient.

<sup>b</sup>Same in CombiVacS trial (Spain)<sup>15</sup>.

**Table 2.** Brief review of heterologous prime–boost shots

Author(s)	Country	N enrolled	Schedules (n), heterologous boost	Schedules (n), homologous boost	Study design	Age range (in years)	Randomization	Outcome measures	Key findings
<b>Heterologous priming</b>									
Shaw <i>et al.</i> , 2021 <sup>14</sup>	UK	463	Schedules (n), heterologous priming AZ/BNT, 4w interval (n = 110)/BNT/AZ, 4w interval (n = 114)	Schedules (n), homologous priming AZ/AZ, 4w interval (n = 112)/BNT, 4w interval (n = 117)	Study design Multicentre, participant-masked, RCT	Age range (in years) 50–69	Yes	Outcome measures Initial reactogenicity and safety	Key findings Greater systemic reactogenicity with both heterologous boosters than their homologous counterparts
Benning <i>et al.</i> , 2021 <sup>46</sup>	Germany	166 HCWs	AZ/BNT (n = 35)	BNT/BNT (n = 82) AZ/AZ (n = 17)	Prospective, single-centre, observational cohort study	26–60	No	Reactogenicity and immunogenicity in Homologous ChAdOx1 nCoV-19 or heterologous ChAdOx1 nCoV-19/BNT162b2 vaccinated	Heterologous ChAdOx1 nCoV-19/BNT162b2 was safe and induced strong and broad humoral response.
Hillus <i>et al.</i> , 2021 <sup>47</sup>	Germany	380	AZ/BNT, 10–12w interval (n = 104)	AZ/AZ, 10–12w interval (n = 38) BNT/BNT, 3w interval (n = 174)	Prospective observational cohort study	28–59	Yes	Assessment of reactogenicity and immunogenicity of heterologous ChAdOx1 nCoV-19 (AstraZeneca) and BNT162b2 (Pfizer-BioNTech) compared with homologous BNT162b2 and ChAdOx1 nCoV-19	Heterologous ChAdOx1 nCoV-19–BNT162b2 well tolerated and improved immunogenicity compared with homologous ChAdOx1 nCoV-19 and BNT162b2.
Schmidt <i>et al.</i> , 2021 <sup>48</sup>	Germany	110	AZ/RNA, interval not specified (n = 54)	AZ/AZ, 9–12w interval (n = 26) RNA/RNA, 3–6w interval (n = 26)	Prospective study	50.6 ± 11.9	No	SARS-CoV-2-specific T cells and antibodies analysed in transplant recipients and controls after homologous or heterologous shots.	Heterologous vaccine induced antibodies and CD4 T cells and seemed promising in transplant recipients.
Groß <i>et al.</i> , 2021 <sup>49</sup>	Germany	26	AZ/BNT, 8w interval (n = 26)	BNT/BNT, unclear interval (unclear n)	Observational study	25–46	No	Evaluation of solicited adverse reactions, humoral and cellular immune responses with ChAdOx1 nCoV-19 prime and BNT162b2 boost	Heterologous ChAdOx1 nCoV-19 prime, followed by BNT162b2 boost, was safe and effective, providing flexibility for future vaccination strategies, especially during shortages.
Normark <i>et al.</i> , 2021 <sup>50</sup>	Sweden	88 HCWs	AZ/MOD, 9–12w interval (n = 51)	AZ/AZ, 9–12w interval (n = 37)	Prospective cohort study	23–62	No	Clinical study of longitudinal immunogenicity of vaccines	The mRNA-1273 vaccine stimulated SARS-CoV-2-specific B-cell memory generated by a prime dose of ChAdOx1 nCoV-19 and protected against the B.1.351 variant than ChAdOx1 nCoV-19 boost.
Dimeglio <i>et al.</i> , 2021 <sup>51</sup>	France	132 HCWs	≤55y: AZ/BNT, unclear interval (n = 33) > 55y: AZ/BNT, unclear interval (n = 22)	≤55y: BNT/BNT, unclear interval (n = 22) AZ/AZ, 8–12w interval (n = 226)	Prospective cohort of seronegative HCWs	20–75	No	Determination of neutralizing antibodies using a live virus-based assay	Stronger antibody response elicited with ChAdOx1-S/BNT162b2 heterologous regimen among HCWs older than 55 years than either of the homologous regimens.
Borobia <i>et al.</i> , 2021 <sup>52</sup>	Spain	676	AZ/BNT, 8–12w interval (n = 450)	AZ/AZ vaccine, 8–12w interval (n = 226)	Phase 2, open-label, randomized, controlled trial	18–60	Yes	Immunogenicity and reactogenicity of BNT162b2 second dose primed with ChAdOx1-S	Mild or moderate reactions such as injection site pain, induration, headache, and myalgia were reported; no SAEs were reported.
Barros-Martins <i>et al.</i> , 2021 <sup>53</sup>	Germany	1493 HCWs	AZ/BNT, 6–12w interval (n = 55)	AZ/AZ, 9–12w interval (n = 32)/BNT/BNT, 3–4w interval (n = 46)	Prospective, observational study	21–64	No	Frequencies and phenotypes of spike-specific T cells	Similar S-IgG, S-IgA, and variant-specific nAbs in AZ/BNT and BNT/BNT

(Continued)

Table 2. Continued

Author(s)	Country	N enrolled	Schedules (n), heterologous boost	Schedules (n), homologous boost	Study design	Age range (in years)	Randomization	Outcome measures	Key findings
Vallée <i>et al.</i> , 2021 <sup>54</sup>	France	197 HCWs	AZ/BNT, 12w interval (n = 130)	BNT/BNT, 4w interval (n = 67)	Retrospective, cross-sectional, monocentre study	>18	No	Assessment of immunogenicity of BNT162b2 (Pfizer/BioNTech) the second dose primed with ChAdOx1-S (AstraZeneca)	Heterologous and homologous ChAdOx1-S and BNT vaccines elicited immune responses after the second shot.
Fabricius <i>et al.</i> , 2021 <sup>55</sup>	Germany	144	AZ/BNT, 12w interval (n = 26) AZ/Mod, 12w interval (n = 10) AZ/BNT, 9–12w interval (n = 572) BNT/AZ, 9–12w interval (n = 167) AZ/BNT, 10–11w interval (n = 11)	1 x AZ/AZ/AZ AZ/AZ, 9–12w interval (n = 461) BNT/BNT, 9–12w interval (n = 113) AZ/AZ, 24–64 (mean 41) AZ/BNT, 27–56 (mean 46)	Observational cohort study Database survey	19–73 18–75	No No	Comparison of immunological responses with BNT162b2 and ChAdOx1-nCoV-19 Reactogenicity	Enhanced protection against SARS-CoV-2 with heterologous vaccinations and boosters using mRNA vaccines Higher reactivity was seen in heterologous immunization with mRNA or adenoviral-vector vaccines
Behrens <i>et al.</i> , 2021 <sup>57</sup>	Germany	23	AZ/BNT, 28d interval BNT/AZ, 28d interval	AZ/BNT, 28d interval BNT/AZ, 28d interval	Prospective cohort study	24–64	No	Analysis of plasma from ChAdOx1-S-primed vaccines after homologous ChAdOx1-S or heterologous BNT162b2 boost3 to compare neutralizing activity against SARS-CoV-2	All heterologous ChAdOx1-S/BNT162b2 vaccinated individuals achieved at least 2.5% neutralization titre against all variants, including the delta variant.
Liu <i>et al.</i> , 2021 <sup>58</sup>	UK	830	AZ/BNT, 28d interval BNT/AZ, 28d interval	AZ/BNT, 28d interval BNT/AZ, 28d interval	Participant-blinded, randomized, non-inferiority trial	50.1–69.3	Yes	Safety and immunogenicity of heterologous ChAd and BNT vaccines	Four SAEs were reported across all groups, but none related to immunization.
Yorsaeng <i>et al.</i> , 2021 <sup>59</sup>	Thailand	214	SINOVAC/AZ, 4w interval (n = 54)	SINOVAC/SINOVAC, 3w interval (n = 80) AZ/AZ, 10w interval (n = 80)	Cross-sectional serological study	2–78	No	Evaluation of immune response	Combination of different available vaccines warranted
Schmidt <i>et al.</i> , 2021 <sup>60</sup>	Germany	216 immunocompetent individuals	AZ/RNA, 9–12w interval (n = 96)	AZ/AZ, 9–12w interval (n = 55) RNA/RNA, 3–6w interval (n = 62)	Observational study	40.8 ± 11.1	No	Reactogenicity	Heterologous boost well tolerated and comparable to homologous mRNA boost. Taken together, heterologous vector/mRNA boost induced humoral and cellular immune responses.
Brehm <i>et al.</i> , 2021 <sup>61</sup>	Germany	873 HCWs	AZ/RNA	AZ/AZ RNA/RNA	Longitudinal cohort study	30–49	No	Assessment of SARS-CoV-2 seroconversion and vaccine-induced immunity	Higher anti-S1-RBD SARS-CoV-2 antibody titres with heterologous prime-boost of AZD1222 followed by an mRNA vaccine indicated higher efficacy than homologous shots.
Gram <i>et al.</i> , 2021 <sup>62</sup>	Denmark	5,542,079	AZ/RNA (n = 1,365,510)	N/A	Nationwide population-based cohort study	33–55	No	Estimation of vaccine effectiveness on combining ChAdOx1 first dose and mRNA vaccine second dose	Reduced risk of SARS-CoV-2 on combining ChAdOx1 and mRNA vaccine, compared with the unvaccinated
Hammerschmidt <i>et al.</i> , 2021 <sup>63</sup>	Germany	85	AZ/BNT, 2–3 m interval (n = 54)	AZ/AZ, 2–3 m interval (n = 31) BNT/BNT, 3w interval (n = 30)	Prospective cohort study	N/A	Yes	Assessment of plasma after ChAd priming and after homologous ChAd or heterologous BNT prime-boost to neutralize the Delta variant	Heterologous ChAd/BNT vaccination led to a 9-fold increase in neutralizing titres, whereas homologous ChAd boost slightly increased neutralization of Delta variant.
Havervall <i>et al.</i> , 2021 <sup>64</sup>	Sweden	2149 HCWs	AZ/BNT (n = 116)	BNT/BNT (n = 67) AZ/AZ (n = 82)	Observational, single-centre study	31.75–51.25	No	Determination of IgG and Nab against SARS-CoV-2 following two-dose with BNT162b2 (BNT/BNT), ChAdOx1 (ChAd/ChAd), or heterologous ChAdOx1 followed by BNT162b2 (ChAd/BNT)	Persistent neutralization of Alpha and Delta variants after infection may aid vaccine policymakers in prioritizing vaccine supply.
Rose <i>et al.</i> , 2021 <sup>65</sup>	Germany	59	AZ/RNA	AZ/AZ BNT/BNT	Observational study	18–56	No	Comparison of anti-S and anti-RBD IgG response after heterologous immunization with a SARS-CoV-2 vector prime and an mRNA boost to that with homologous shots.	Administration of a vector vaccine followed by an mRNA boost resulted in a humoral immune response, comparable to that after two mRNA vaccinations.
Skowronski <i>et al.</i> , 2021 <sup>66</sup>	Canada	380,532 specimens	Mixed RNA AZ/RNA	RNA/RNA AZ/AZ	Test-negative designs	18–80+	No	Comparison of two-dose vaccine effectiveness by mRNA and/or ChAdOx1	Two mRNA and/or ChAdOx1 shots provided persistently protection against Delta variant at least for 5–7 months post-vaccination.

(Continued)

Table 2. Continued

Author(s)	Country	N enrolled	Schedules (n), heterologous boost	Schedules (n), homologous boost	Study design	Age range (in years)	Randomization	Outcome measures	Key findings
Tenbusch <i>et al.</i> , 2021 <sup>67</sup>	Germany	480	AZ/BNT1, 9–12w interval (n = 232/250 <sup>†</sup> )	AZ/AZ, 9w interval (n = NA/66*) BNT/BNT, 21d interval (n = 410/250*) * cohort A/cohort B	Non-blinded, non-randomized study	N/A	No	Quantification of antibody response in vaccines with heterologous ChAdOx1 nCoV-19 prime and BNT162b2 mRNA (BioNTech-Pfizer) boost Safety and immunogenicity of heterologous prime-boost shots of BBV152 (Covaxin) and AstraZeneca's ChAdOx1-nCoV-19 (Covishield)	Heterologous shot induced higher neutralization than homologous ChAdOx1 nCoV-19 or homologous BNT162b2.  No major SAEs; reactogenicity with heterologous shots showed that mixing of two vaccines derived from different platforms was safe. Heterologous shots improved protection against variants of concerns (VOCs) to overcome challenges of shortage of any vaccine.
Kant <i>et al.</i> , 2021 <sup>68</sup>	India	98	AZ/Covaxin (n = 18)	N/A	Observational study	54.25–69.75	No		
<b>Heterologous boosting</b>									
Author(s)	Country	N enrolled	Schedules (n), heterologous boost	Schedules (n), homologous boost	Study design	Age range (in years)	Randomization	Outcome measures	Key findings
Amar <i>et al.</i> , 2021 <sup>69</sup>	USA	458	JAN + MOD (53) JAN + BNT (53) 2 x MOD + JAN (49) 2 x MOD + BNT (51) 2 x BNT + JAN (51) 2 x BNT + MOD (50) 2 x SINOVAC + AZ (n = 210)	JAN + JAN (50) 2 x MOD + MOD (51) 2 x BNT + BNT (50)	Non-randomized CT (Mix-and-match study)	19–85	No	Safety, reactogenicity, and humoral immunogenicity on 15 and 29 days	Reactogenicity similar to primary series; no vaccine-related SAEs. Homologous and heterologous boosters were well-tolerated and immunogenic in adults
Yonsaeng <i>et al.</i> , 2021 <sup>70</sup>	Thailand	549	N/A	N/A	Observational study	40–48	No	SARS-CoV-2 spike receptor-binding-domain (RBD) IgG, anti-RBD total Ig and antispike protein 1 (S1) IgA	High immunogenicity of AZD1222 booster after completion of two-dose inactivated vaccines
Li <i>et al.</i> , 2021 <sup>71</sup>	China	300	2 x SINOVAC + CANSINO (95 PP) 1 x SINOVAC + CANSINO (49 PP) 2 x SINOVAC + BNT (27)	2 x SINOVAC + SINOVAC (100 PP) 1 x SINOVAC + SINOVAC (49 PP)	Observer-blind RCT	18–59	Yes	Neutralizing antibodies against live SARS-CoV-2 at 14 and 28 days after the booster dose	No SAEs; heterologous boost associated with more frequent AEs (particularly injection-site pain), but generally mild/moderate
Keskin <i>et al.</i> , 2021 <sup>72</sup>	Turkey	69 HCWs	2 x SINOVAC + BNT (27)	2 x SINOVAC + SINOVAC (18)	Observational study	41 ± 10.9	Yes	To investigate the interplay between humoral immune responses	IgG-N titres for both groups showed statistically significant differences (both p-values < 0.001)
Moghnieh <i>et al.</i> , 2021 <sup>73</sup>	Lebanon	125	2 x SINOPHARM + BNT (50)	N/A	Pilot prospective cohort clinical study	18–75	No	Humoral immunity induced by a single dose of BNT162b2 compared to that produced by two BNT162b2 doses	BNT boost safely and well-tolerated
Patamatamkul <i>et al.</i> , 2021 <sup>74</sup>	Thailand	41 HCWs	2 x SINOVAC + BNT (n = 23) 2 x SINOVAC + AZ (n = 18)	N/A	Observational study	32.04–38	No	Antibody response among those boosted with BNT162b2 or ChAdOx1 nCoV-19	High reactogenicity for both boosters
Huat <i>et al.</i> , 2021 <sup>75</sup>	Singapore	115	JAN + BNT (14)	JAN + JAN (28)	Observational study	23–75	No	Spike-specific humoral and cellular immunity in Ad26.COV2.S vaccinated those who were primed with Ad26.COV2.S only, or boosted with a homologous (Ad26.COV2.S or heterologous (BNT162b2) second dose.	Heterologous vaccination enhanced Spike-specific humoral and cellular immunity in Ad26.COV2.S vaccinated
Sablerrolles <i>et al.</i> , 2021 <sup>76</sup>	Netherlands	434 HCWs	JAN + MOD (n = 112) PP) JAN + BNT (n = 111 PP)	JAN + JAN (n = 106 PP)	Participant-blinded, multi-centre, RCT	18–65	Yes	Immunogenicity and reactogenicity of homologous and heterologous boosters in Ad26.COV2.S-primed	Boosting of Ad26.COV2.S-primed well-tolerated and immunogenic

## Characteristics of Ideal Heterologous COVID Vaccines

An ideal SARS-CoV-2 vaccine would elicit a protective response within one month of administration, and would develop the cell mediated, antibody mediated immune and mucosal immune responses in effective level with minimum adverse effects. Such response should remain for a minimum of 6 months after one or two vaccinations and should protect older adults and the immunocompromised.<sup>11,44</sup> Additionally, it should be manufactured on a large scale.<sup>31</sup> Previously, adenoviral vectors were combined with DNA and poxviral vectors to enhance cellular and humoral immunity. However, homologous adenoviral regimens were not preferred due to the reduced potency of the second dose due to anti-vector immunity.<sup>44</sup>

## Prospects to the Heterologous Prime–Boost COVID Vaccines

Both Com-Cov2 and CombivacS trials have tested heterologous combinations of only 4 and 2 COVID vaccines, respectively, developed with three platforms—VVnr, mRNA and PS. However, trials of vaccines developed in seven other platforms are still pending (Table 1). Also, from the preliminary data generated, it is unclear whether mild-to-moderate VAEs are due to prime dose or heterologous boost. Until and unless all combinations are trialed in a diverse population, it would be premature to declare them safe and efficacious, and such trials will demand longitudinal studies. Therefore, we have to rely on the data generated from the ongoing currently. The heterologous prime–boost schemes for COVID-19 vaccinations have been included in the national vaccine policy of some countries while others are considering it. Brief review of heterologous prime–boost shots is presented in Table 2.

## Conclusions

Heterologous prime–boost approaches, after considering the candidate vaccines' platforms, number needed to vaccinate and intervals, adjuvanted status, excipients and preservatives added, efficacy and effectiveness, vaccine adverse events, and boosters, have shown safe and effective outputs among humans, proving the same as a milestone in vaccination campaigns, the primary benefit being the uninterrupted rollout and supply chain despite problems in one or two vaccine shots. Although large-scale controlled trials with all available permutations of COVID-19 vaccines are warranted to make the findings generalizable and applicable in the broader perspective, the beneficial results with heterologous approach in terms of improved immunogenicity, reactogenicity, safety, effectiveness and flexibility made it an alternative to the practitioners and policymakers globally.

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## Contributors

B.S. conceptualized, performed literature review, drafted and revised the manuscript; Bh.S., R.S., D.A., R.S., S.S. and A.J.R.M. contributed to the literature review and critically revised the manuscript. All authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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